The Journal of Parasitology

Volume 10 MARCH, 1924 Number 3

STUDIES ON THE LIFE HISTORY OF *TRICHOSOMOIDES CRASSICAUDA* (BELLINGHAM) *

LYELL J. THOMAS

Trichosomoides crassicauda (Bellingham), a nematode parasitic in the urinary bladder of rats, especially *Epimys norvegicus*, has remained one of the oddities of the animal world in that the male of the species is parasitic within the uterus of the female. Aside from this peculiar habit on the part of the male, little has been known regarding the life history. The writer after a study of the anatomy of the male began a series of experiments on the mode of infection in rats. Important facts have come to light that are of general as well as special interest in view of the findings in the same as well as in other families and genera by Yokogawa (1921), Yoshida (1919), Fülleborn (1920), Ransom (1922), and others. This investigation was begun at the suggestion of Dr. H. B. Ward to whom the writer wishes to express his sincere appreciation.

REVIEW OF LITERATURE

Bellingham (1840) first discovered and named the species of this nematode as being common in the urinary bladder of the Norway rat for which the name *Epimys norvegicus* (Erxl.) will be used in the discussion throughout. Rayer (1843) reported the same worm in rats and gave it the name of *Trichosoma muris decumani*. Stossich (1890: 18) in his monograph cites Bayer (1843), which undoubtedly is a typographical error. The classification and external characters of the female were noted by Dujardin (1845), Diesing (1851), and Eberth (1863). No report of the male was given until Walter (1866) discovered the dwarfs in the uterus of the females which he thought to be very large young, while it remained for Leuckart (1867*a*) to recognize their true nature. However, A. Schmidt sent Leuckart some dwarf males which possessed a copulatory apparatus and reported having found a very few occasionally beside females in the urinary bladders of rats. Leuckart was inclined to believe a dimorphism existed between

* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 234.

the males in the uterus without a copulatory apparatus and these external forms possessing a sheath and spicules. Von Linstow (1874) determined the males discovered by Schmidt as a new species, which he named *Trichosoma schmidtii,* and those discovered by Walter as *Trichodes crassicauda,* but unfortunately the genus Trichodes was preoccupied. He gives a possible mode of infestation together with a very incomplete anatomy of the male, but makes no mention of the more careful work of Bütschli (1872). . It was suggested that the eggs containing embryos, after being passed in the urine, are ingested by the rats in contaminated food or water. In the stomach of the host the shell is opened and the embryos escape to bore into the digestive tract and make their way to the pelvis of the kidney possibly by way of the blood stream, particularly the renal artery. Females sexually undeveloped and little larger than males were found in the pelvis of the kidney beside males. Copulation was suggested as taking place in the ureters, the males from three to six entering the vagina and passing up the uterus where they turn about so that the anterior end is directed towards the vaginal opening. Eventually the females come to lodge in the urinary bladder. In 1882 von Linstow discovered a stylet in the newly hatched larva.

Löwenstein (1910: 535-44) while investigating the formation of tumors in the urinary tract of rats, found eggs containing embryos imbedded in the pelvis of the kidney. Serial sections showed parasites to be present in the renal vessels, free or imbedded in tissue or in the kidney capsule or ureters. Adult parasites were reported in the tissue of the kidney pelvis, the fat capsule of the kidney and some distance from the kidney in the tissue of the ureter. Frequently he found free Trichosomes beside blood vessels, with a scattering of red blood corpuscles near them, so he concludes that the youngest stages wander in and out of the capillaries of the bladder wall into the tissue; they move freely in the tissue, which accounts for the rule in observation that the eggs and young forms come from the kidney through the ureters to the bladder. He suggests that infection possibly takes place by the ingestion of the eggs and that the larvae find their way to the bladder, kidney, ureters, etc., by way of the blood stream. In his work (1911 a: 767), as evidence for a direct infection by way of contaminated food, he found that "fresh" rats became infected when placed in cages that had contained infected rats when no sterilization precautions were observed, also through the contaminated hands of an attendant who fed both infected and uninfected animals. A dog and a mouse kept in the same room and fed by the same attendant had characteristic papillomas of the bladder which he believed would have shown the presence of *T. crassicauda* had he taken the time to examine them carefully. Following this same article his work (1913) is questioned by Fibiger. Löwen-

stein gives no data on his "fresh" rats as to how determinations were made that they were not previously infected. Fibiger found 48 out of 64 wild rats examined infected with *T. crassicauda* in the urinary bladder, and of 55 mottled and white rats from the laboratory stock 47 were infected with the same parasite.

Hall (1916) observed embryos escape from their egg shells in the vagina of the female after the worm had been in normal salt solution a brief period. From this, and the fact that the embryos lived only a little while, he concluded that infection must take place in a rather short time.

The first real attempt at experimental infestation was made by Yokogawa (1921), who reported the occurrence of the larvae in the body cavity, pleural cavity, and lungs of an experimental white rat fed with large numbers of eggs and adult worms. From this experiment it was concluded that in this species the larvae migrate directly through the body tissues and cavities of the rat into the lungs, where they must spend a part of their life cycle. Further discussion of the literature and its bearing on the results of the present paper will be left until after the presentation of the experiments.

Briefly a summary of synonymous names for the parasite are as follows: *Trichosoma crassicauda* Bellingham, 1840; *Trichosoma muris decumani* Rayer, 1843, also Bayer (of Stossich, 1890, misprint for Rayer, 1843); *Tricocephalus crassicauda* Eberth, 1863; *Trichodes crassicauda* (Bellingham, 1845) von Linstow, 1874; *Trichosomum crassicauda* (Bellingham) Bütschli, 1872; *Trichosoma crassicanda* Bütschli, 1873, (misprint for *crassicauda*); *Trichosoma crassicaudatum* Leuckart, 1867 (apparently for *crassicauda*); *Trichodes crassicauda specifica* Löwenstein, 1910 (apparently for crassicauda); *Trichosomoides crassicauda* (Bellingham, 1840) Railliet, 1895; Hall, 1916 (type *crassicauda*).

Regarding the systematic relation of *Trichosomoides crassicauda* to other members of the family *Trichinellidae,* some interesting points will be dealt with later in the discussion.

METHODS

Eggs were collected by the following methods:

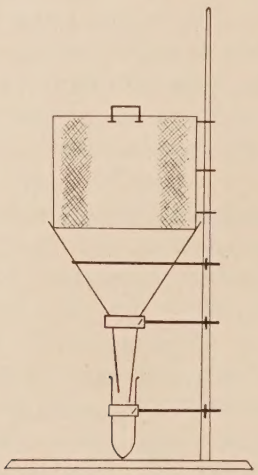
1. Large filter papers placed under the cage were of advantage in showing distribution of eggs normally passed in the urine but not practicable for obtaining a supply for experimental purposes.

2. Glass plates were placed under the cages, and the urine collected, together with the eggs, formed a crystalline deposit after evaporation. Such eggs were used in experiments testing the viability of embryos after exposure to the air for some time.

3. A large glass funnel fitted under a round cage and emptying into a large centrifuge tube was found to be most efficient in determining the presence of mature eggs passed in the urine of the confined rat, as well as obtaining a small supply of eggs for experiments (Text fig. A).

4. For mass infection experiments the necessary recourse for obtaining a sufficient quantity of eggs was to kill numbers of wild rats and get from these freshly killed animals what parasites and eggs were contained in their bladders. Eggs and larvae were examined in normal salt solution, and by the Looss (1911) glycerine-alcohol method.

The following methods were used to trace the migrations of larvae within the host. After chloroforming the experimental animal the abdominal and pleural cavities were opened as quickly as possible and each washed out with 20 c.c. normal salt solution, great care being



Text Figure A.—Arrangement of ring stand, round cage, funnel, and centrifuge tube for collecting eggs from urine.

taken to avoid opening blood vessels, and to keep pipettes and centrifuge tubes for the two cavities separate. Blood was drawn from the portal, renal and postcaval veins, also from both ventricles and auricles of the heart, with syringes containing a few c.c. of 2 per cent. sodium citrate solution to prevent coagulation. The blood was washed three or four times in normal salt solution by means of the centrifuge and then examined for larvae. The solutions from the body cavities were centrifuged once and examined. In some cases, the body cavity solutions were placed in Syracuse watch crystals and examined after standing a few minutes. One-half the viscera (kidneys, liver, spleen and lungs) were crushed through a 120-mesh copper screen together with normal salt solution, centrifuged, and the sediment

examined, a modification of the method used by Yoshida (1919). The other half of the material was killed in hot Bouin's, sectioned 6 to 10μ, and stained with Delafield's hematoxylin and eosin. Serial sections, 10μ thick, were made through the entire thorax and abdominal region of a young infested rat.

Infestations were obtained by feeding to white and wild rats, previously determined as uninfested, fresh eggs and adult worms in bread crumb pellets moistened with normal salt solution. The albino rats were obtained from disease-free stock. The wild rats, *Epimys norvegicus,* were taken from the nest when about three weeks old, judging from their size, and reared in captivity for over two months before being used for experimental purposes. With the exception of very young animals whose parents had been determined as free from the parasite, the urine of all animals was examined for the presence of eggs from time to time over the period of a month or more to eliminate the possibility of any previous infection. Animals were also exposed to experimentally infested stock, to unsterilized cages and air-dried eggs and worms.

The diet of the experimental animals consisted of oats, corn, buckwheat, hempseed, sunflower seed, dog-biscuit, fresh vegetables, and table scraps. To preclude infestation by means of the grains purchased in the market which might have been subject to contamination from wild rats, the experiments testing the viability of eggs exposed to air for some time were carried on. Also those experiments in which uninfested animals were placed in cages not sterilized after having been used by infested animals. All other cages were thoroughly autoclaved and in the case of the round cages used with the funnel, flaming was used to destroy all trace of eggs which might adhere to the wire.

BIOLOGY OF HOST AND PARASITE

Before considering the experiments, let us examine the conditions under which the parasites exist in nature, their reported occurrence, and degree of infestation within the wild rat. The frequenting of moist places, dark underground burrows, chance contamination of food stored by rats in such places, together with their licking habits, all may contribute as factors in autoinfection and the distribution of the parasite among rats.

Most observations show that the worms frequent the urinary bladder, although von Linstow (1874), Löwenstein (1910), Saul (1914), and the writer find various stages through the urinary tract, though not so frequent nor in such numbers in the kidney capsule, pelvis of the kidney and ureters. Some authors give the liver and other organs of the rat as the habitat of the parasite. Such situations are not the normal

habitat of the worm and the author is inclined to believe that trichosomes other than *T. crassicauda* were mistaken for the parasite.

The worm has been reported wherever the urinary tract of *Epimys norvegicus* has been examined; thus in Ireland, England (London), Germany (Offenbach), Denmark (Copenhagen), Austria (Trieste, Rätzelburg), France (Paris), Australia (Brisbane, Sydney), United States (Washington, D. C.; Lincoln, Nebraska; San Francisco, California; Urbana, Illinois).

Johnston (1918) found six worms in the urinary bladder of only one out of 163 specimens of *Epimys rattus* or a 0.6 per cent. infestation. Of 246 *Epimys alexandrinus* examined only one contained *T. crassicauda* and that a single specimen. Ship and house rats, varieties of *Epimys rattus* showed a 0.5 per cent. infestation of 409 specimens. Seventy-four out of 205 *Ep. norvegicus* were parasitized making a 31.2 per cent. infestation. He also notes that the greatest number of females obtained from any one host was 15 (twice), 11, 10 (twice), and 9 (several times). The usual number was from one to three. Of 83 mouse bladders examined, none possessed the parasite.

Balfour (1922) in his observations on the wild rats in England found *T. crassicauda* (Bell.) in the urinary bladder of 48.3 per cent. of 333 brown rats examined.

Löwenstein (1910) in his experiments on the formation of tumors in the urinary tract of rats used 54 rats from four different sources. In the first series of 18 rats all 18 were parasitized; 16 times the worms were found in the bladder, once in the kidney and once in the kidney and ureters. A second series of 18 rats showed papillomas of the bladder in four, and in five only a simple proliferation of the epithelium and urethra. No lesions were noted in nonparasitized animals. In 9 rats of the third series examined, 5 had parasitized bladders, four of which had papillomas. Concerning the pathological conditions caused by the parasite, more will be said later about the observations made by the writer.

From time to time over a period of four years, the author has examined the urinary tract of numbers of rats from Champaign and Urbana, Illinois, and vicinity. Of 136 adult rats examined 102 were parasitized; 357 parasites were taken. The average number of parasites per host was 3.5 or a 75 per cent. infection. Thirteen half-grown rats averaged 1.5 parasites per host. In all, 149 rats contained 360 worms or a 69.8 per cent. infection with 3.4 parasites per host. The male rats far outnumbered the females taken as only 33 adult females were trapped, of which 20 were infested. The average number of parasites per female was 3.3 per host. Out of 103 adult males, 82 were infested, giving a 79.6 per cent. infection with an average of 2 parasites per host.

Leuckart (1867a) in 250 rats found 2000 worms which, even granting all 250 were infested would give a rather high number of parasites per host. The heaviest infections found by the writer were 12 (four times), 11, 10, and 21 in single hosts. This last number, 21, is the heaviest infection on record and is possibly due to mass infection from the eating of other heavily parasitized rats. In Exp. 17, the largest number of parasites, 14, was recorded for a mass infection by laboratory methods. Although no records were made of the extent of the infection through the summer months, the writer has noted on two occasions when rats were examined in midsummer that the parasite was present in about the same numbers as shown for the average infestation found in the fall, winter, and spring. There is no great seasonal change in the infection as was found by Veglia (1915) in *Haemonchus contortus* (Rud.), dependent upon external factors for its development. The observation first noted by Hall (1916) that the larvae emerged from the shell while in the uterus of the female if placed in normal salt solution, and the fact that the larvae soon died under those conditions might lead one to conclude that the period of infestation is short. This same phenomenon was observed by the writer and will be dealt with later. The work of Yokogawa (1921) indicated that the infection might be direct. An adult rat once infested may be capable of reinfecting itself and perhaps by chance those rats with which it associates.

In this investigation the experimental rats were kept in a live room at normal room temperature but the humidity was probably greater than that of the average room because of the presence of large open aquaria full of water; the light was also subdued, hence simulating conditions approaching those frequented by *Epimys norvegicus* under natural conditions. A variety of foods were fed from grains and dog biscuit to table scraps. Five rats from the same laboratory stock as the experimental animals, kept under the same conditions but not exposed to infested animals or food, were used as controls. Ten rats were used in the completed infestation experiments and exposed by several different methods to the parasite.

EXPERIMENTAL WORK

Eggs of *T. crassicauda* when mature are of a golden brown color, capped at both ends with a not prominent opercular plug (Fig. 5). According to the measurements of various observers they vary considerably in size and shape ranging from 62 to 72μ in length and from 29 to 56μ in width, and from a spheroid to almost an oblong in outline.

However, at the proper angle they may be seen to be dished in on two sides. The openings in the opercular cap measure slightly over 8μ in diameter, just large enough to allow the fully developed but flexible

embryo to squirm through at hatching. The writer has seen embryos pushing with their stylets against the opercular plug and inferred that the hatching must be an active process on the part of the embryo as well as caused by external agencies. The observations made upon hatching in normal salt solution lead one to conclude that in such solutions it is perhaps due to differences in osmotic pressure, for under such conditions embryos have been seen to suddenly slip out of the egg tail first, as though the opercular plug suddenly gave way under pressure and the end nearest the opening, in this case the tail end, was forced out. In the experiments on the hatching the writer obtained an egg from the stomach of the rat in Exp. 31, which had a living embryo within it after having been in the rat stomach for two and a half hours (Fig. 1); pressure of the cover slip caused the posterior end suddenly to shoot out and a granular and membranous mass followed such as was found adhering to a normally hatched egg taken from the stomach of rat Exp. 32 (Fig. 5). This material extruded from the egg upon the hatching of the embryo, the writer has interpreted as waste products of metabolism, together with the remains of the vitelline membrane. The egg has three principal membranes; first, the outer shell, staining black with Hiedenhain hematoxylin or ironalum; second, an almost blue-gray fertilization membrane, and third, a brownish vitelline membrane, as shown in figure 5*a*. Between the vitelline and fertilization membranes is found the peri-vitelline space. The shell consists of two rather distinct layers, the outer rugose and the inner of almost transparent consistency; the entire shell is about 0.6μ, whereas the shell and membranes together are 2μ thick. Such a thickness of the shell is not what one would expect in an egg which is viable only a short time outside the host. On the contrary, it has all the characteristics of an egg that may withstand desiccation and other external conditions to which it might be subjected.

Immature eggs do not have the golden brown color, although the embryos within them seem mature and active. When eggs are laid a sticky substance is secreted causing them to adhere more or less together in stringy masses. The eggs themselves seem adhesive and will stick to glassware and other smooth objects when moist. Filter papers placed under the rat cages showed that as a rule eggs are passed out and distributed in bunches. Probably this is due to the secreted substance which some authors have interpreted as extra shell material; however, there are definite glands (Fig. 29) for some distance posterior to the vulva and on the dorsal surface that may be concerned with this secretion; but of these more will be said later.

The fact that eggs are passed off in masses would seem to be a measure that better insures numbers being licked up at one time by the

host. In mature worms from ten to twenty or more golden brown eggs are present in the vaginal region and hundreds in various stages of development extend on back into the uterus. In cases where the vaginal opening is imbedded in the tissue of the host, eggs may be found extending from the vagina back to the point where the posterior end of the worm protudes into the urinary lumen and both eggs and worm surrounded by a cuticula-like sheath. One series of experiments show that the eggs are fairly resistant to drying in the air which further insures their being ingested by the host with contaminated food.

Attempts were made to determine where the eggs hatch out in the host and the length of the incubation period within the host with the following results:

Exp. 1.—Gastric juice extract was made from rat stomachs and an adult female worm with numbers of eggs adhering to her, was placed in the fresh extract at 11:15 a. m., October 30, 1922, at 37° C. Results: After three hours no action was observed either on the worm or eggs and at 9 a. m., October 31, 1922, the worm was dead and no larvae had hatched.

Exp. 2.—A wild rat which had just been fed some oats was killed November 25, 1922, and one adult female *T. crassicauda* was taken from the urinary bladder. The stomach was cut out and the worm inserted within it at 10 a. m.; the open ends were ligatured and the stomach placed in normal salt solution at 37° C. until 2 p. m. when it was opened and examined. Results: The worm was partly macerated. A rent in the uterus allowed the eggs to flow out into the stomach contents and numbers of embryos came out of the eggs almost immediately. These newly hatched larvae began to actively crawl about in the stomach contents. One larva was observed penetrating tissue by revolving movements of the body with the stylet end pressing against the tissue.

Exp. 3.—To confirm the above experiment, another wild rat was allowed to go without food for a time and at 9 a. m. on November 27, 1922, about 20 gm. of bread were eaten by the animal. At 10 a. m. the rat was killed, the stomach removed, and three adult female worms with quantities of eggs adhering to them were introduced into it. The cardiac and pyloric ends were ligatured and the stomach placed in normal salt solution at 37° C. Results: At 1 p. m. the stomach was opened and the macerated parts of two worms were found but none of the eggs seemed to have hatched. The stomach content was of a creamy consistency while that of the stomach in Exp. 1 was brownish green. It was suggested that bile might be the activating substance necessary to induce hatching.

Exp. 4.—Numbers of mature eggs were placed in normal salt solution February 14, 1923, at 3:15 p. m., and observed for a time under a binocular microscope. Results: After five minutes a few larvae had hatched. These were fairly active and pressed against the bottom of the watch glass with their anterior ends as if seeking a point for penetration. At 8 a. m., February 15, 1923, the larvae were still alive, though very sluggish. When examined an hour later, all were dead.

Exp. 5.—Eggs were placed in normal salt solution at 37° C. on February 14, 1923. Results: At 3:15 p. m. a few larvae hatched out after about five minutes and were dead after thirty minutes. All embryos within the eggs were dead after three hours at 37° C. Some of the newly hatched larvae and unhatched eggs were kept at 37° C. on a warming stage in normal salt solution and a solution of crushed and filtered rat liver added. The larvae were

dead after a few minutes and the eggs showed no signs of hatching after three hours. In the same way a 0.2% HCl solution was added to another set of eggs without any results.

Exp. 6.—A wild rat was killed February 14, 1923, and the stomach removed at 3:15 p. m. An adult worm and quantities of eggs were inserted within it, the open ends of the stomach were then ligatured and the whole placed in normal salt solution at 37° C. Result: At 4:15 p. m. and 6:15 p. m. no action was observed either on the worm or eggs.

Exp. 7.—A few larvae from Experiment 4 were placed on epithelium from the rat's stomach according to the Goode (1922) method and observed for one hour, by which time all were dead. None of them seemed to have enough energy to puncture the tissue, although two were noticed attempting it by placing their heads with the stylet against the tissue and twisting their bodies about spirally, but did not penetrate beyond the slime or mucous coat on the epithelium.

Exp. 31.—On February 21, 1923, at 2:20 p. m. a urinary bladder with three worms imbedded in it and quantities of mature eggs adhering to it, was ingested by an adult albino rat. Results: The rat was chloroformed and examined at 4:20 p. m., just two hours after the ingestion of the worms and eggs. The stomach contents showed the urinary bladder undigested in the cardiac end and the partially digested free posterior ends of the three females. Numbers of eggs were found throughout the stomach contents, all contained larvae and apparently none had hatched. The small intestine showed no trace of larvae or eggs. No larvae were found in the body cavities, portal vein, liver, lungs, kidneys or spleen.

Exper. 32.—An adult albino rat was placed in a cage without food over night and the following morning at 8:05 a. m., February 23, 1923, it was fed a bread pellet containing two adult worms and quantities of eggs together with a urinary bladder covered with eggs. At 11:15 a. m., three hours later, the animal was killed and the stomach contents examined. Results: The partially digested bladder was found at the pyloric end of the stomach. Empty egg cases were found in the stomach contents such as is shown in Figure 5, measuring 68 to 44μ. Granules and a hyaline membrane were present at the end from which the larvae had escaped. The same was noted adhering to a newly hatched larva as in Figure 1.

Although no definite conclusions may be drawn, it would appear that a slight stimulus is necessary to cause the larvae to hatch, in that by simply placing the eggs in normal salt solutions, some do hatch and may remain alive as in Exp. 4 at room temperature for 17 hours, others may die in a few minutes, particularly those kept at 37 C. Differences in osmotic pressure between the egg contents and salt solution may be the direct cause of the larvae leaving the egg. Although no larvae were seen to penetrate completely into tissue, attempts were noticed in the foregoing experiments. Later experiments show that larvae must actually penetrate the tissues of the host. The incubation period in the rat stomach is somewhere in the neighborhood of three hours as indicated by Exp. 31, 32, and subsequent experiments in another series.

Desiccation and Viability of Eggs

Exp. 18.—December 1, 1922, twenty adult *T. crassicauda* with many mature eggs in their uteri were mixed with moist bread and allowed to dry at room temperature (70° F.). On December 5, at 5:30 p. m., these dried worms were

fed. Results: Repeated examinations of the urine failed to show any trace of *T. crassicauda* eggs. April 18, 1923, at 3 p. m., the animal was chloroformed. The left lung had tumor-like growths imbedded in it. A large club-shaped cystic tumor filled the contracted bladder. The small end plugged the entrance of the right ureter into the bladder.

Exp. 19.—Mature eggs from several wild rat bladders were collected so that large numbers were obtained. These were mixed with moist bread crumbs and the pellet thus made was allowed to dry in the air at room temperature (70° F.). At 5:30 p. m., December 5, 1922, the eggs were fed to the rat. Results: Examinations from time to time of the urine failed to show the presence of eggs, the last examination being on January 12, 1923, at 9 a. m., the rat having been confined in the cage over the collecting tube throughout the night. No more examinations were made until April 18, 1923, when at 8 p. m. the rat was chloroformed. The bladder contained three adult females and numbers of eggs.

The presence of a papilloma in Exp. 18 would seem to point to an infection of *T. crassicauda,* for all observed papillomas from wild rats are associated with the worm, the majority of the worms being imbedded in the tumor itself. This is also in accord with the observations of Löwenstein (1910) and Saul (1914). Experiment 19 shows plainly that desiccation for five days does not destroy the viability of the embryos within eggs mixed with food materials.

Infestation by Associations

Exp. 25.—A half-grown albino female rat in heat at the time was placed on January 16, 1923, at 10 a. m., with the wild male of Exp. 17. Results: On February 8, twenty-two days after being exposed to the wild male, a urine examination showed no eggs present. Again on February 23, 37 days after the exposure, no eggs were present. March 6 seven young were born, all of which were eaten by the mother. On April 19, at 10 a. m., the rat was chloroformed and three adult females with numbers of eggs were found adhering to the bladder wall.

Exp. 26.—A half-grown albino female rat was placed on February 5, 1923, at 8 a. m., with the male rat of Exp. 2. The female was not in heat at the time. Results: On February 23, no eggs were found in the urine, eighteen days after the exposure. The male was killed February 17, so the two had associated together for about 12 days. On April 19, the female was chloroformed and no worms found.

Exp. 27.—A half-grown albino male rat was placed with the male of Exp. 6 at 8 a. m., February 5, 1923. On February 14, the male from Exp. 6 was removed but the same bedding and feed dish were left in the cage. Results: On April 4, fifty-eight days from the time of first exposure, no eggs were passed in the urine. April 19 the rat was chloroformed and a young female, not as yet laying mature eggs, was taken from the right ureter near its opening into the bladder. Two adult worms having laid dozens of mature eggs were all completely imbedded in a large papilloma of the bladder wall.

Exp. 28.—At 8 a. m., February 5, 1923, a half-grown albino female rat in heat at the time, was placed in the same cage with the adult male of Exp. 15. Results: On February 28, eight young were born and all but one eaten by the mother. This young one was saved for future experiments. The female was chloroformed on April 20, and one adult worm was found adhering to the bladder. Numbers of mature eggs had been laid.

Since three out of four rats exposed to infested animals became infested, it may be inferred that rats in nature in close association with one another may spread the infestation by this means.

THE LARVA

Embryos of *T. crassicauda* when ready for hatching are commonly coiled in a figure eight and in the brownish shelled eggs do not seem nearly so active as those in the uncolored egg cases, and are not so liable to be released in normal salt solution. As with the egg, there is considerable variation in the size of embryos, ranging from 390 to 264μ in length, from 8 to 12μ at the anterior end, from 10 to 16μ at the widest part, and from 6 to 8μ wide at the posterior end. The average total length is around 297μ, and the average width at the anterior end is about 10μ, which corresponds very closely with measurements given by von Linstow (1882). Löwenstein (1911a) gives a length of 400μ, yet his measurements of eggs are not greater than those given by other observers. The embryo when ready to hatch is equipped with a stylet at the anterior end which is thrust in and out against any surface

TABLE 1.—*Measurements in Microns of Living Larvae Just Escaped from the Egg*

Total Length	Width at Anterior End	Width at Middle	Width at Posterior End
264	12	16	8
390	10	14	8
303	10	14	8
315	10	12	8
265	10	10	8
294	8	10	6
310	8	12	6
281	12	12	6
273	8	10	6
275	8	10	6
297	10	12	7

with which it may be in contact and measures about 8μ in length. Clear hyaline granules may be seen in the posterior part which is more or less attenuated, but truncated at the end (Fig. 1). At the tip of the anterior end is a more hyaline region surrounding the stylet, around which the cuticula is seen to roll up like a prepuce (Fig. 3) and may be compared somewhat with that of the blood inhabiting *Microfilaria bancrofti*. Such an embryo has a more or less straight digestive tract running its entire length, the esophagus of which is tripartite in cross section (Fig. 3*a*). In longitudinal section through the mid region of the intestine a single layer of cells forms its walls (Fig. 3*d*). About 4μ back from the anterior end is a clear space which does not stain readily and as in the larva (Fig. 28) stained in 5 per cent. formalin with Delafield's hematoxylin, there is a more or less absence of nuclei. This region corresponds very closely with that in the male and female where a nerve ring appears to be present (Figs. 6, 15). No large esophageal cells are present but in a larva taken from the abdominal cavity one to seven

days after the ingestion of eggs by a white rat, cells that appeared to be beginning esophageal cells are evident (Fig. 2).

Numerous attempts were made to determine the wanderings of the parasite in the body of the host.

Exp. 3.—A half-grown albino male rat from a hooded mother was left without food or water for 24 hours, then fed one adult *T. crassicauda* with many eggs in a bread pellet at 11 a. m., October 27, 1922; again at 6 p. m. five adult worms and quantities of eggs were fed. Two days later, October 29, 1922, at 3:30 p. m., twelve adult worms were fed in two bread pellets, together with a urinary bladder to which numbers of eggs were adhering. The following day at 2:15 p. m., a quantity of eggs were again fed in two bread pellets. The animal appeared normal and was given a few sunflower seeds. At 3 p. m., October 31, approximately 4, 2, and 1 days after the various feedings, the rat was chloroformed and examined. Results: On October 28, a few empty egg cases were found in the feces, indicating that the larvae must have hatched. At 8 p. m., the animal appeared sick, being humped up, its coat ruffled, and ears, feet and tail waxy white.

The examination on October 31 was a typical examination for this series and was conducted as follows: the abdominal and thoracic regions were quickly skinned. A small incision was made in the abdominal wall and 10 cc. of normal salt solution was injected by means of a pipette several times and removed again by the pipette. The washings from the cavity were then placed in a centrifuge tube and later centrifuged. Care was taken in this operation not to open blood vessels and bring about a possible contamination.

As much blood as possible was removed by means of a syringe from the portal vein and a 2 per cent. sodium citrate solution added to prevent coagulation. This was also saved for centrifuging. The lung cavity was opened and washed out, the fluids being saved for examination. Blood was drawn from the right ventricle by means of another syringe as from the portal vein. Many bleeding points were noticed over the surface of both lungs, also on the tip of the right liver lobe. The left lung, the heart, both kidneys and ureters, the bladder and right lobe of the liver were saved for sectioning. The rest of the liver and right lung were separately crushed through a fine sieve with normal salt solution and the resulting fluids centrifuged.

Four larvae were thus found measuring about 298μ in length from the crushed liver; four larvae were found in the abdominal cavity washings measuring from 280 to 298μ in length; six larvae measuring 253 to 273μ in length were found in the pleural cavity washings; two larvae were found in the centrifuged fluid from the crushed lung and measured about 253μ in length. The above measurements made from living material vary with the contraction and elongation of the larvae. Those in the abdominal cavity averaged around 6μ in width at the anterior ends and 4μ at the posterior ends. Those in the pleural cavity ranged from 6 to 14μ at the anterior ends and from 4 to 7μ at the posterior ends. Larvae taken from the right lobe of the liver measured the same at anterior and posterior ends as those taken from the abdominal cavity. The two larvae taken from the left lung measured about 14μ at the anterior ends and about 7μ at the posterior ends; they were exceedingly active, as were those from the liver. The sluggish movements of the larvae from the abdominal cavity were very pronounced in contrast; their stylets moved in and out as if probing their surroundings; great numbers of leukocytes were also present. Great care was taken to keep the washings from the body cavities and organs separate. No larvae were found in the portal or heart's blood nor in the sections of organs saved. In all, sixteen larvae were recovered from this experimental animal.

Exp. 12.—On November 16, 1922, a half-grown albino female rat was fed a quantity of eggs and two adult worms in two bread pellets at 11 a. m. Again

on November 20 at 11 a. m., two more worms and a quantity of eggs were fed in bread pellets. Two days later at 10:30 a. m., four adult worms and quantities of eggs were fed. Results: November 18, 1922, two days after the first feeding of worms and eggs, the rat appeared very sick and seemed to have difficulty in breathing. On November 23, the rat was chloroformed and examined at 2 p. m., 8, 3, and 1 days after the various feedings. The animal appeared very weak and anemic when killed.

The abdominal cavity washings contained three larvae; these larvae when examined on a slide exhibited boring actions and were very active. They measured about 8μ at the anterior end, 10μ through the midregion, and tapered towards the ends which were 6μ wide. No larvae were found in the lung cavity, liver, portal vein or right ventricle. The left lung was very congested and one larvae with the following measurements was taken from it; a larva measured at the anterior end 10μ wide, midregion 10μ, posterior end 6μ, total length 372μ. This larva was very active; its stylet moved in and out pushing against the slide. Only these four larvae were recovered from this animal.

Exp. 30.—An adult female albino rat was given no food or water over night and at 9:30 a. m., February 17, 1923, was fed in a bread pellet the urinary bladder from rat Exp. 2, which was covered with *T. crassicauda* eggs. Water was given the animal but no more food. Five hours after the ingestion of the eggs at 2:30 p. m., the rat was chloroformed and examined. Results: Two very active larvae were recovered from the abdominal cavity. The right lung showed several bleeding points, also a few were noticed on the right lobe of the liver and on the right kidney; these together with the spleen, urinary bladder, and stomach were fixed in 5% formalin. Blood drawn from the right auricle and vena cava contained nine larvae.

Exp. 39.—A young albino female rat 15 days old was given by means of a pipette in a drop of normal salt solution eight adult worms that were first decapitated, also quantities of eggs, at 8 a. m., March 17, 1923. Ten hours later at 6 p. m., the animal was chloroformed and quickly skinned. Small incisions were made in the thorax and abdominal cavity to allow rapid action of the hot Bouin's killing fluid. After the final imbedding in 62° C. paraffin, transverse sections 10μ thick were made through the thoracic and abdominal regions. Results: Sections thus far have shown empty egg cases in the stomach and small intestines and larvae in the bronchi.

Exp. 20.—On December 5, 1922, at 5 p. m., fifty eggs were placed in a bread pellet and fed to a half-grown male albino rat. Results: Approximately five days later, on December 10 at 11:10 a. m., the rat was discovered dead, being still warm. An examination was made at once. Only one larva was found and that in the lung cavity. It measured 231μ long, 10μ wide at the anterior end, 12μ in the midregion, and 6μ at the posterior end. A few bleeding points were noted on the liver.

Exper. 24a.—The same rat used for Exp. 24 was fed 11 adult worms and numbers of eggs in a bread pellet at 11:55 a. m., January 29, 1923. At 10 p. m., approximately ten hours after the ingestion of eggs, the animal was chloroformed and examined. Results: One very active larva was found in the abdominal cavity. Blood drawn from the right ventricle contained six larvae. One measured 239μ long, 6μ at the anterior end, 4μ at the posterior end, and 70μ back from the anterior end it was 8μ wide.

Completed Infestations

Exp. 2.—At 3 p. m. on October 23, 1922, a half-grown male albino rat was fed twelve adult worms in two bread and hamburger pellets, together with a urinary bladder to which a quantity of eggs adhered, taken from a wild rat. Again on October 24, numbers of eggs were fed in two bread pellets. The following day the animal was placed on the regular diet. Results:

Frequent examinations of the urine were made but no eggs found until December 9, forty-seven days from the date of the first feeding. It is probable that eggs were present in the urine much earlier than this but were not found because of imperfections in technique. On December 14, the penis of this rat was first noticed remaining protruded and although its appetite was good, its coat was long and rough and the animal did not grow. On December 17, it was chloroformed and the parasites used for Exp. 30. Eight adult female *T. crassicauda* were found in the urinary bladder with males in their uteri and masses of eggs containing living larvae. All of the females were attached by their anterior ends to the epithelial lining of the bladder.

Exp. 4.—On November 2, 1922, at 9 p. m., a half-grown male albino rat was fed about fifty eggs in water and on carrots. Quantities of eggs adhering to a urinary bladder removed from a wild rat were fed again on the 9th at 10 p. m. The following day at 5:30 p. m. another urinary bladder together with a quantity of eggs in a bread pellet was fed. Results: Frequent examinations failed to show eggs in the urine until December 9; with improvement in the technique numbers of eggs were found 37, 30, and 29 days respectively after the ingestion of eggs. On December 19, the animal was chloroformed. A young male (Fig. 18-24) parasite was found free in the lumen of the left ureter near its entrance into the bladder, a more detailed account of which will be given later. One young female was found also in the left ureter but near the pelvis of the kidney with no male in her uterus but a young male beside her. Three young females were found imbedded in the tissue capsule of the right kidney. They also were without males in their uteri, and as yet no eggs were found developing. In the right ureter two young females were seen through the wall inside the lumen with two males near them. An adult female, with a male in her uterus together with mature eggs, was found imbedded by the anterior end in the bladder wall. A young female with immature eggs and a male in her uterus was attached to the bladder wall at the point where the left ureter empties into it. In all, six males and eight females were found.

Exp. 6.—A half-grown female albino rat was fed an adult worm together with a quantity of eggs in a bread pellet at 10 p. m., November 9, 1922. Again on November 19, one adult worm and numbers of eggs were fed in a bread pellet at 5:30 p. m. On November 15 one more adult worm and quantities of eggs were fed in a bread pellet at 7 p. m. Results: Eggs appeared in the urine 30, 29, and 24 days respectively after the ingestion of worms and eggs; although frequent examinations were made it was not until December 9 that the technique was more perfect, consequently as in the previous experiments it is possible eggs were present in the urine at an earlier date. On December 15, 1922, the penis was noted to remain protruded, the rat did not grow, and its coat was rough. On January 14, 1923, the animal was etherized; the body cavities were washed out; blood was drawn from the portal vein and right ventricle, and the body organs were examined by sectioning and maceration methods. The left lung showed a large bleeding spot and was saved for sectioning as well as the heart, spleen, right lobe of the liver and the right kidney and ureter. Seven adult female worms with males in their uteri and great quantities of eggs were found in the urinary bladder. One free male was found in the bladder contents.

Exp. 9.—Fifty eggs were fed to a half-grown male albino rat on November 16, 1922, in two small bread pellets, 18 in one and 26 in the other, at 3:30 p. m. Results: On December 10, 1922, at 10 a. m., just 24 days after the ingestion of the eggs, one egg was found in the urine, the shell was mature, but the contents a shapeless mass. Another egg had a fully developed larva. Several *Syphacia obvelata* were passed in the feces. At 8 a. m., December 18, 1922, the animal was found dead and internal organs beginning to distintegrate. No *T. crassicauda* were found but numbers of *Syphacia obvelata,* both males and females, were found in the caecum.

Exp. 13.—An old adult albino male rat was fed at 11 a. m., November 20, 1922, with a bread pellet containing three adult worms and a quantity of eggs. Again on November 22 at 10:30 a. m., four adult worms and numbers of eggs were fed as before. Repeated examinations failed to show any *T. crassicauda* present but numbers of *Syphacia obvelata* were passed in the feces.

Exp. 15.—Fifteen adult worms in a bread pellet were fed at 5:30 p. m., December 5, 1922, to an adult albino male rat. Results: An examination was made, 14 days after the ingestion of the eggs, but no eggs were found in the urine. No further examinations were made until January 13, 1923, at 1 p. m. when dozens of *T. crassicauda* eggs were found in the urine, 39 days from the time eggs were ingested. Four *Syphacia obvelata* were passed in the feces. The animal was chloroformed February 21, 1923. Three adult worms with males in their uteri were attached to the bladder wall. Masses or rather strings of eggs were laid and were adhering to the parasites as well as the bladder wall. These worms and eggs were used in Exp. 31. A young female was taken from the lumen of the right ureter near its entrance to the urinary bladder; immature eggs were present in the uterus, but no male. This female possessed a stylet as is the case with the larvae.

Exp. 17.—A young wild rat was removed from the nest October 3, possibly three weeks old at the time, judging from its size. It was reared in captivity and frequent examinations made of the urine. On December 5, 1922, at 5:30 p. m., three adult worms and a quantity of eggs were fed in a bread pellet. Results: No examination of the urine was made until January 16, 1923, 42 days from the time the eggs were ingested, when numbers of eggs were found. The 26th of January at 8 a. m. the rat was chloroformed and twelve females with males in their uteri were removed from the urinary bladder. Only three were laying mature brown shelled eggs. These were used for Exp. 33. The left ureter near its entrance into the pelvis of the kidney contained in its lumen a young female with a male in her uterus together with an abundance of eggs. However, the eggs had not the characteristic brownish shells of the mature eggs found in the bladder, but they did contain living embryos. The right ureter contained one young female near the pelvis of the kidney with no male in the uterus but a few eggs were beginning to form and in the posterior part of the uterus, the vitelline membrane was laid down on some but the contents were only a shapeless mass. This female was partially imbedded in the wall of the ureter and a stylet was present.

Exp. 21.—A young wild rat, *Epimys norvegicus*, was taken from the nest when about four weeks old October 3, 1922, and kept in captivity. Frequent examinations of the urine showed no *T. crassicauda* eggs to be present. On December 16, 1922, at 11 a. m., five adult worms together with quantities of eggs were fed in a bread pellet. Results: The animal was chloroformed and examined January 8, 1923, at 2 p. m. Three adult females with males in their uteri were found attached to the bladder wall. Quantities of mature brownish shelled eggs were laid which were adhering to the worms and bladder. All of these females were examined for stylets but none were in evidence. A young female removed from the lumen of the right ureter near its entrance into the bladder, however, possessed a stylet but no male was in her uterus. A few eggs with polar caps formed were present in the uterus and averaged 57 by 23μ. Just 23 days had elapsed since the ingestion of eggs.

Exp. 22.—Another wild rat from the same litter as the animal of Exp. 21, reared and observed in the same manner, was fed five adult worms together with a quantity of eggs in a bread pellet at 11 a. m., December 16, 1923. Results: On January 9, 1923, at 1 p. m., the animal was chloroformed and examined, twenty-four days after the ingestion of eggs. Three adult females, their uteri filled with eggs and masses adhering to their bodies were found attached to the urinary bladder. All three had one or more males in their uteri.

Exp. 24.—A female albino rat 62 days old was fed six adult worms together with a quantity of eggs in a bread pellet January 12, 1923, at 9 p. m. On the following day, January 13, at 3:45 p. m., four adult *T. crassicauda* together with a quantity of eggs were fed in a bread pellet. Two days later, January 15, at 1:30 p. m., seven adult *T. crassicauda* together with a quantity of eggs were fed in a bread pellet. On the 16th, the rat appeared slightly sick and seemed to have difficulty in breathing. At 11:55 a. m., January 29, 1923, eleven adult worms and eggs were fed in a bread pellet to furnish data for another set of experiments, hence the results are not noted in this experiment. Results: No larvae of a size commensurate with the number of days elapsed (15, 17, and 18 days) were found after the animal had been chloroformed January 30, 1923, at 10 a. m., and the organs and cavities of the body examined.

In the following table the writer has summarized all of the foregoing experiments on the length of time required for eggs to appear in the urine after the ingestion of eggs. The finding of young individuals in organs other than the bladder is not always the reward of diligent search, neither does the absence of parasites noted indicate that no parasites may be present.

TABLE 2.—*Period of Development of Larvae to Egg-Laying Females*

No. Exp.	Date of Infection	Days Elapsed Until Eggs First Noted in Urine	Days Elapsed Until Bladder Examined	No. of Parasites Found ♂	No. of Parasites Found ♀	Location of Parasites
2	10/23/22	47	117	..	8	Urinary bladder
	10/24/22	46				
4	11/ 2/22	37	47	..	2	Urinary bladder
	11/ 9/22	30	40	3	2*†	Right ureter
	11/10/22	29	39	..	3*†	Kidney capsule
6	11/ 9/22	30	97	..	8	Urinary bladder
	11/10/22	29	96			
	11/15/22	24	91			
9	11/16/22	24	32	0	0	
13	11/20/22	..	152	0	0	
	11/22/22					
15	12/ 5/22	39	78	..	3	Urinary bladder
17	12/ 5/22	42	83	..	1†	Left ureter
					1*	Right ureter
					9†?	
					3	Urinary bladder
21	12/16/22	23	23	..	1*†	Right ureter
					3	Urinary bladder
22	12/16/22	24	24	..	3	Urinary bladder
24	1/12/23	18	18	0	0	
	1/13/23	17	17			
	1/15/23	15	15			

* Where males are free in the rat they are included in the count; all females are counted and if not indicated with an asterisk possess males in their uteri.
† Females not laying eggs.

SUMMARY OF ALL COMPLETED INFESTATIONS

Experiments 21 and 22 show that correlated with the appearance of eggs in the urine, adult egg laying females may be expected in the bladder in approximately three weeks after the ingestion of eggs. The

presence of other individuals in other parts of the urinary tract, as the young female in the right ureter of Exp. 21, seems to indicate a retardation in the development as well as the progress of some of the individuals within the host. This individual possessed a stylet which is a larval characteristic, as no adult female found in the urinary bladder has been found with a stylet. Although all males within the uterus of females that were examined possessed stylets, other factors may enter in to cause their retention. The presence of a stylet and its significance will be discussed later. No male was in the uterus of this young female although there were a few immature eggs in the uterus. In some, the polar caps had begun to form and contained within the shell irregular masses of granular material as of disintegrated cells. Two such eggs measured 56 by 22μ and 58 by 24μ. The measurements of this young female were as follows: width at the anterior end 28μ, length of the esophagus 1540μ, width at the vulva 70μ, length of intestine 6580μ, width at the posterior end 84μ, total length 8120μ.

In Exp. 4, at approximately seven weeks from the first ingestion of eggs, two adult females were in the urinary bladder, two young females and two young males in the right ureter, having not as yet copulated, and three immature females in the capsule of the right kidney, the *tunica fibrosa*. Measurements for two of these females from the tunica fibrosa were as follows: width at the anterior end 28μ for both, length of the esophagus 810μ and 1540μ, width at the vulva in both 56μ, length of intestine 1540μ and 4886μ, both were 42μ wide at the posterior end; total lengths 2350μ and 6426μ. The presence of these young individuals in this situation in this instance might represent a secondary infection as well as a retardation of individuals developing from the eggs ingested at the known periods. However, the presence of younger individuals in the tunica fibrosa, renal pelvis, and ureters, than in the urinary bladder is significant and points to the path of migration in the host, more about which will be said later.

That the ingestion of fresh eggs may be a method whereby infections are obtained in a fair percentage of cases is shown by the results of the experiments. It is interesting to note the coincidence in the percentage of infestation; a 70 per cent. infestation was obtained by feeding eggs to the experimental rats while the total infestation of all wild rats examined was 69.8 per cent. Experimentally by mass infestation the average number of parasites per host was nearly doubled, 6.7, as compared with the average number, 3.4, in wild rats under natural conditions. Of course, it is realized that the number of experimental rats is too small to make any sweeping conclusions as to how infestations occur in wild rats, but it does not seem impossible that ingestion of fresh eggs is the normal method in that the experimental rats were

so easily infested by this means. Concerning also the possibilities of infestation by worms or eggs exposed to the air for some time will be dealt with in a later series of experiments.

CONTROLS

The five control rats kept throughout the experiments were killed and examined on April 21, 1923. No parasites were found to be present.

THE MALE AND FEMALE

The male worms (Fig. 18-24) first discovered by Walter (1866) and later described by Bütschli (1872) and von Linstow (1874) possess larval characteristics which these earlier observers overlooked, also other anatomical features which the writer has thought best to describe in their proper place. The adult worms vary between 1600 and 3478μ in total length, according to measurements made by the writer; however, according to Löwenstein (1911a) males were found measuring 5,200μ in length; Stossich (1898), Leuckart (1867), von Linstow (1874), Bütschli (1872), Hall (1916) give 2,500μ as the average length. The greatest body width which generally occurs in the region of the juncture of the intestine with the esophagus (Fig. 20) varies between 19 and 40μ. Other authors have found variations between 23 and 33μ.

The length of the esophagus varies between 702 and 1280μ with a corresponding difference in the number of esophageal cells from 48 to 81 in specimens counted. In the esophageal cells of the male, a great molecular activity was noticed. As the lumen in the esophagus opened and closed in peristaltic waves beginning at the anterior end and darting back, a similar but slower peristaltic movement occurred along the entire length of the esophageal cells in which clear vacuole-like spaces appeared, the tonoplasts (Figs. 11, 12, 17 and 31) indicating great metabolic activity. This active movement in the esophageal region was noted also in young females. Some of the esophageal cells seem almost spongelike in consistency and others adjacent to them are darker and more solid in their cytoplasm; such a relationship is shown in the longitudinal section through the esophagus of a female (Fig. 31) using a Heidenhain iron-alum hematoxylin stain. In the preparation of toto mounts these lighter cells of sponge-like consistency very often collapse.

The function of these cells is problematical and may be connected with digestion. However, they are not directly connected with the esophagus, which runs beneath them and is not entirely enveloped with the esophageal cells in a common membrane as given by Hall (1916) as one of the diagnostic characters of the superfamily *Trichinelloidea*. Neither does it lie embedded within the cell as described by von Linstow

(1897) for *Trichosoma contortum,* with a round capillary chitinous tube, nor as Rauther (1917-18) depicts in *Trichocephalus crenatus* with a common membrane surrounding both the esophageal cells and the tripartite esophagus. It is therefore distinct in itself and does not conform in this particular to the diagnostic characters given for the superfamily *Trichinelloidea.* Not until more of the morphological structures of this entire group have been studied can their true systematic relationships be established. The proposal of Ward (1917) to group the whipworms together in a suborder, the *Trichosyringata,* having as a diagnostic feature a row of esophageal cells pierced throughout their entire length by a delicate tube of minute caliber, in contrast with nematodes having an esophagus tripartite in cross section, the *Myosyringata,* does not conform to these more recent investigations.

No cuticular ridges (Fig. 12-30) have been noted in the male as are found in the female, but minute transverse striations at first described by Bütschli (1872) are present over the entire surface of the body with the exception of the very tip of the anterior end, where a trace of a prepuce (Fig. 33) as is found in larvae, may be seen with the proper staining and illumination. Another larval characteristic found in males taken from the uterus of females as well as from the tissues of the host, is a stylet. Fülleborn (1920: 345) reports the occurrence of a stylet in the larvae of *Trichinella spiralis,* and in the larvae of *Trichuris trichiura* a figure is shown of such a structure. The presence of a stylet in these three forms is of phylogenetic interest in view of the finding of similar structures by Bastian (1864) among free living nematodes. Fülleborn (1920: 347) suggests that perhaps there is a relation between these forms and such nematodes possessing a stylet as *Dorylaimus* and *Tylenchus* which are partly free-living and partly parasitic. Fuchs (1915) has established the existence of such forms in the vagina and uterus of insects in their parasitic state. Perhaps here is an intermediate form between free nematodes and such parasitic ones as *Trichosomoides crassicauda* (Bell.).

In all adult egg-laying females situated in the region of the bladder, the writer has been unable to demonstrate the presence of a stylet. On the other hand all males examined from the uteri of such females possessed stylets. Perhaps it may be inferred from this and other facts that the males lead a more roving life than the females, in the sense that the females settle down and often papillomas completely surround them. The writer has observed males with their posterior ends protruding from the vaginal orifice and twice have males been observed to come out of the vagina into the normal salt solution in which the females had been placed. The writer has also been able to confirm the observations of Bütschli (1872) who before von Linstow (1874)

noted that males turn about in the uterus of the female so that their anterior ends face the vagina. Bütschli (1872), as well as the writer, has seen males headed towards the uterus. In examining the urinary bladders of numbers of wild rats freshly killed, males have been occasionally found free in the urine.

The seminal vesicle of the male does not store spermatozoa in any great numbers and the indications are that the spermatozoa pass gradually into the cloaca and then to the outside through the anus. This compares favorably with the condition in free nematodes.

The seminal receptacle of the female is small and relatively few eggs are found there awaiting insemination at any one time. However, the sac-like uterus becomes distended with eggs in various stages of development. The small seminal receptacle and the relatively few eggs contained in it at any one time awaiting insemination also compares favorably with free nematodes.

Small gland like structures (Fig. 25 *rc*) along the rectal cloacal region of the male, very like those found in *Trichina spiralis,* are suggestive of similar structures in Dorylaimus.

PATHOLOGY

In Experiments 5, 7, and 8, four days after the feeding of adult worms and eggs the young rats died apparently from pulmonary troubles; their lungs were very much congested. Experimental rats 10 and 11 died with similar congested lungs two days after taking adult worms and eggs. Experimental rat 16 died the day after taking three adult worms with its food; its lungs were also congested. Fifty eggs were fed to the young rat in Exp. 14 and seven days later it died with congested lungs. The rat in Exp. 9 died from an unknown cause thirty-two days after receiving fifty eggs with food. No parasites were found in any of these young rats. The young rat in Exp. 20 died five days after the eating of fifty eggs; one larva was recovered from the pleural cavity.

It is evident that *T. crassicauda* may be the indirect cause of deaths among young rats, possibly by the distribution of bacteria sown along the trail of wandering larvae. The researches of Romanovitch (1912) have shown that *Trichina spiralis* distributes bacteria along its way as it passes through the intestinal mucosa. Löwenstein (1910-13) and Saul (1914) found that papillomas are often associated with the worms in different parts of the urinary tract. Although from time to time in the past four years tumors have been noted, no accurate data were kept until January 22, 1923. Out of forty-six wild rats examined eight were found with papillomas of the bladder and ureters. In all cases

adult female worms were imbedded in the tumors. Among the experimental rats No. 18 had a large cystic tumor partially plugging the entrance to the right ureter, 133 days from the first infection. The rat in Exp. 27 had two adult female worms completely enveloped in a large papilloma of the bladder.

Beside the occurrence of papillomas, pulmonary troubles, and bleeding points noticed on lungs, liver, and kidneys in experimental rats, some animals remained dwarfed in size, their coats roughed, and in a general anemic condition. In the series of experiments on completed infections, rat Exp. 2, fifty-two days from the first infection, was found with its penis protruding, seeming unable to retract it within its sheath. More or less inflammation was present. This same pathological condition occurred in rat Exp. 3, thirty-five days from the first infection.

Connected with the occurrence of papillomas in rats, the writer finds in most cases worms actually imbedded or partially enveloped in this epithelial proliferation. At the point where the worm is imbedded in the host, glands (Fig. 29-30) occur in the adult females. Löwenstein (1910) suggested that the presence of papillomas might be due to mechanical stimulation of the worms as well as to some toxin secreted by them. Eberth (1863) first noted beadlike projections on female worms which he believed to be solid bodies (Taf. VII. fig. 9). The writer is of the opinion that he here represents the cuticular ridges (Fig. 30) as shown in cross section or lateral view. Bastain (1864: 556) says regarding similar structures, "What he (Eberth) considers solid staff-shaped prolongations I believe to be integumental channels, similar to those which I have previously described as so common in free Nematoids." Bütschli (1872) was the first to observe the secretion of a sticky substance from the region of these projections, and this the writer has been able to confirm. Bütschli, also confirmed by Jägerskiöld (1901), believed this substance acted to anchor the worm in the tissues of the host. Hall (1916, Fig. 12) has shown the true external appearance of these structures. As the writer has mentioned before there is a secreted sheath surrounding the worm imbedded in the host tissue. As shown by figures 29 and 30 there are definite glands opening to the exterior. It is not improbable that here may be the source of a toxin-like substance suggested by Löwenstein (1910) which is one of the agencies in the formation of papillomas.

Another interesting feature which was suggested by Exp. 13 but which the writer was unable to confirm for lack of material, is that of age immunity. This aged rat was fed seven adult worms and quantities of eggs but did not become infested.

DISCUSSION

Brumpt (1921) is of the opinion that the circulatory system is only a secondary path for the migration of larvae, for in a series of experiments, he found that by placing nematode cultures of *Strongyloides vituli* in the buccal cavity of mice, nematodes that had shown no specificity or attraction for penetration of the umbilical cord of the calf, traversed the buccal mucus and the esophagus, and came into the pyloric region of the stomach. Within two hours the mouse died and showed larvae in the mediastin, diaphragm, ocular lobes, and brain.

The work of Yoshida (1919) would seem to support the opinion of Brumpt (1921). Yoshida found Ascaria larvae in the pleural cavities of eight out of ten guinea-pigs killed and examined twenty to seventy-five hours after the feeding of eggs, eleven being the largest number recorded. He isolated larvae from the livers of other guinea-pigs killed forty-eight to seventy-five hours after feeding with Ascaris eggs and injected them into the abdominal cavities of nine guinea-pigs. These animals were examined less than twenty hours up to sixty-six hours after injection and a few larvae were recovered from the pleural cavity, and in some cases many were found in the lungs. Also in two cases where larvae had been injected into the pleural cavity of guinea-pigs and the animals examined after death which occurred in less than twenty hours, a few larvae were found. From this, Yoshida (1919:24, 25) believed that although a few larvae might have reached the lungs from the abdominal cavity by passing through the liver and heart by way of a blood vessel, the majority get into the lungs by boring through the diaphragm. He has concluded that larvae after hatching out in the intestine pierce through its walls and come into the abdominal cavity. From here they migrate in all directions, some coming into the liver, spleen, pancreas, or kidneys and a few finally arriving in the lungs by virtue of their own power to bore through tissues and not by way of blood vessels.

At variance with this view, the work of Ransom and Foster (1920), and Ransom and Cram (1921), without doubt indicates that Ascarid larvae do travel by means of the blood stream and do not regularly bore through the intestinal wall into the abdominal cavity. Stewart (1921) also came to this same conclusion, as did Fülleborn (1920:344) who does not find that dog ascarids penetrate into the abdominal cavity, but finds great numbers of them in the mesenteric lymph nodes. Yoshida (1919) evidently did not carefully examine the blood of his experimental animals. The work of Looss (1911) has shown that among Strongylids such as *Ancylostoma duodenale* Dub. the lymph and blood streams are clearly modes of transporting larvae to the lungs.

Experimental evidence thus far favors the view that among Strongylids and Ascarids, larvae are carried by way of the blood and lymph streams to the lungs where they undergo one phase of their life cycle.

Among the family *Trichinellidae* complete life histories are not so well known. *Trichinella spiralis* copulates in the intestine of the host animal; the male lives but a short time. The female, however, bores into the mucosa and glands of Lieberkühn and the embryos are freed in the lymph spaces. They are finally brought to the heart and scattered throughout the body by means of the blood stream. Some few may wander off into the lungs but the majority come to lodge in the muscles of the host where encystment occurs. Here they remain until freed again by ingestion and the digestion of the cyst in another host animal. Fülleborn (1920: 345) found that *Trichuris trichiura* larvae after the ingestion of eggs by guinea-pigs and rabbits came to lodge in the intestine where they continued their development. He says in substance that although he observed their hatching and beginning development in the cecum nothing in his experiments would indicate that they came into the circulation or lungs. An interesting point in his observations is the finding of a stylet in the larvae of *Trichinella spiralis* and of *Trichuris trichiura,* the latter of which he represents in a figure; the importance of this will be brought up later in this discussion. Yokogawa (1921) reports on a Japanese paper by Neshi (1918) (which the writer unfortunately has been unable to read as yet), who is reported to have found four larvae of *Trichuris depressiuscula* in the lungs of a dog twenty-one hours after experimental infection. This Yokogawa (1921) uses to support his assumption that the family *Trichinellidae* are among those nematodes a part of whose life cycle must be spent in the lungs. It is important to examine Yokogawa's evidence. Three rats were used in his experiments from only one of which were the larvae recovered. In all fifteen worms and quantities of eggs were fed in the various feedings to this one rat. From one to four days had elapsed before the animal was killed and examined. Yokogawa recovered in all nine larvae, four from the abdominal cavity which measured 820 to 840μ in length and 34 to 35μ in width, two from the pleural cavity, and three from the lungs. Two of the larvae from the lungs and the two from the pleural cavity measured about 2340μ in length. The width increased toward the middle of the body to a maximum of 100 to 110μ. One of the larvae found in the lungs was smaller than the other two in the same location, but had a size and structure similar to those found in the abdominal cavity. He inferred the larger larvae to be males and the smaller ones females. It is a very singular circumstance that these larvae found in the lungs and pleural cavity from one to four days

after the various feedings should have such a phenomenal growth. Rapid growth is found among certain genera and species of nematodes. However, from the measurements of the adult males and females as given by different observers, it appears that these so-called larvae surpass many of the males in length and are equal to the width of many adult females which have been found in the urinary tract. The separate sexes of worms with these measurements (2340μ), are easily distinguished in such a situation. No such phenomenal growth as reported by Yokogawa (1921 : 82) was observed in the present experiments.

TABLE 3.—*Measurements of Living Larvae Recovered from Experimental Rats*

Location in Host	No. Days in Host	Total No. Larvae Recovered	Total Length	Width at Anterior End	Width at Middle	Width at Posterior End
Abdominal cavity	4, 2, 1	4	280	6	..	4
			298	6	..	4
			298	6	..	4
			298	6	..	4
	8, 3, 1	3	290	8	10	6
			290	8	10	6
			290	8	10	6
		1				
Average	.....	..	289	6	..	4
Pleural cavity	4, 2, 1	6	273	6	..	4
			253	14	..	7
			253	14	..	7
	5	1	231	10	12	6
Average	.....	..	255	11	..	4
Lung	4, 2, 1	2	253	14	..	7
			253	14	..	7
	8, 3, 1	1	372	10	10	6
Average	.....	..	292	13	..	6
Liver	4, 2, 1	6	298	10	..	6
Ventricle	10 hrs.	6	239	6	8	4
Right auricle	5 hrs.	9				
Total number larvae recovered		39				

All dimensions are given in microns.

In considering next the data on migrations of *T. crassicauda,* one should examine the evidence as shown by the present set of experiments. There were twenty-one experimental animals used in the attempts to determine the various stages of development of the larvae within the host. From seven of these rats thirty-nine larvae in all were recovered; their measurements and distribution are shown in Table III. In Exp. 30 blood drawn from the right auricle and vena cava contained nine larvae; two were taken from the abdominal cavity and one from the pleural cavity, five hours after the ingestion of eggs by an adult rat.

In Exp. 24a six larvae were taken from the right ventricle and one from the abdominal cavity, ten hours after the ingestion of eggs and eleven adult worms by a half-grown rat. Their measurements compare favorably with larvae just hatched. Experiments 3 and 12 in which twenty-two larvae in all were recovered from one to eight days after the ingestion of worms and eggs, there was apparently little if any change in the size of the larvae as compared with larvae just emerged from the egg, Table 1. In these two experiments, fifteen larvae were found in the blood stream, as compared with three taken from the abdominal cavity and one from the pleural cavity. Yokogawa (1921:83) does not mention any examination of the blood, but says, "The eggs swallowed by the final host hatch in the digestive tract and penetrate through its wall into the abdominal cavity. From here they travel into the pleural cavity, probably through the diaphragm and penetrate into the lungs from their surfaces." Since larvae have been found in the blood stream it is more reasonable to assume that larvae come into the pleural and abdominal cavities by chance wanderings and the bleeding points on organs is the external evidence of their leaving the blood stream. Furthermore the author in numerous cases has found blood filled renal tubules in the kidneys of parasitized rats that would indicate larvae had left the blood vessels, and the finding of young forms themselves in blood vessels and renal tubes would bear this out. It might also be well to recall a similar observation made by Löwenstein (1910); he, however, found only young forms beside blood vessels with a scattering of red corpuscles near them. Romanovitch (1912) injected larvae of Trichina into the peritoneal cavities of guinea-pigs and found that after two weeks no larvae could be found either in muscles or in the cavities; therefore he concluded they were completely absorbed. It is not unlikely that this same thing may occur to numbers of *T. crassicauda* that wander out of the blood stream and come to lodge in other positions than the urinary tract which are unfavorable for development. As has been shown before, all larvae possess an active piercing instrument in the stylet, by which they might extricate themselves in such situations and bore their way out through the organ into the body cavity. Yokogawa (1921:82) says regarding the newly hatched larvae, "The larvae just from the eggs have a very small body, of almost uniform thickness, terminating in bluntly rounded ends. They measure about 0.21 to 0.25 mm. in length and 8 to 10μ in thickness. It was impossible to make out any details of internal structure at this stage." A morphological study of the larva at this stage shows it fully equipped with a stylet, esophagus, and an intestine. A few cells that possibly might be the beginning of esophageal cells are shown in Figure 2.

In the opinion of the writer the blood stream is the means of dispersal of *T. crassicauda* larvae in the host. Although no larvae have been found between the eighth and twenty-third days, one must consider other evidence for this view. The writer has recovered larvae from the blood stream in his experiments; also while dissecting a wild rat on March 5, 1923, he found a young female worm without a male in her uterus in a renal blood vessel in the kidney. Again on March 15, another young female was taken from a renal blood vessel in the left kidney of a wild rat. These young females were equipped with stylets. Löwenstein (1910: 543-544) reports the frequent finding of Trichosomes beside blood vessels with a scattering of red corpuscles near them and suggests that the youngest stages possibly wander in and out of capil-

TABLE 4.—*Measurements of Living Immature Females and Adult Males from Experimental Animals*

Sex of Parasite	Location in Host	Total Length	Length of Esophagus	Width at Anterior End	Width at Vulva	Width at Post-Esophageal Region	Length of Intestine	Width at Posterior End
♀	Left ureter	2280	980	28	70	..	1400	56
♀	Kidney capsule	2350	810	28	56	..	1540	42
♀	Kidney capsule	6426	1540	28	56	..	4886	42
♀	Right ureter	8120	1540	28	70	..	6580	84
♀	Bladder	7970	1250	28	98	..	6720	70
♂	Kidney pelvis	2840	1280	22	..	40	1360	20
♀	Renal blood vessel, left kidney	4604	1428	25	50	..	3176	42
♀	Renal blood vessel, left kidney	3020	1260	25	54	..	1764	33
♂	Uterus of ♀ left ureter	2011	856	21	..	25	1155	16
♂	Free in bladder	2450	1190	22	..	28	1260	18
♂	Uterus of ♀ in bladder	3478	1176	22	..	28	2220	18
♂	Uterus of ♀ in bladder	2263	1036	16	..	21	1227	14
♂	Uterus of ♀ in bladder	1600	702	20	..	21	960	13
♂	Uterus of ♀ in bladder	2200	990	24	..	19	1308	13
♂	Uterus of ♀ in bladder	2100	840	24	..	24	1296	18
♂	Uterus of ♀ in bladder	1900	792	16	..	20	1200	13
♂	Uterus of ♀ in bladder	2200	1110	21	..	27	1155	18

All dimensions are given in microns.

laries of the bladder wall into the tissues, and that they move freely in the tissues. That young and adult forms may be scattered through the urinary tract is apparently the case. If one examines Table 5 on the distribution of young forms taken from wild rats, and Table 4 of worms taken from experimental animals, one finds that the distribution of young forms extends from the kidney capsule to the urinary bladder. In heavily parasitized animals, renal tubules are found filled with coagulated blood. The parasite has been found in blood vessels, renal tubes, and lumina of the urinary tract. Although the youngest or least developed forms are more frequent in the vicinity of the kidney, this is as one would expect. The larvae carried by the blood stream circulate freely throughout the body of the host. Some individuals bore their way out into body tissues, and if in the lungs might bore out into the

pleural cavity or bronchi, to perish or wander back into the circulation. Eventually a few come into the filter system, the kidneys, where conditions are suitable for their development. Just what these conditions are will require a greater knowledge of the chemical relations of host tissue and parasite.

Individuals are found that have bored their way into renal tubes, from which situation they can gradually work their way down into the urinary bladder by way of the kidney pelvis and lumen of the ureter, or slowly migrate through tissues from such situations as the kidney capsule. Copulation may take place in all of these situations if the male and female are far enough developed. A young female with a male in her uterus and with eggs containing living embryos was taken from the ureter near its entrance into the pelvis. Löwenstein (1910) found females in a similar situation with eggs. Young females without

TABLE 5.—*Location of Young Parasites in the Urinary Tract of Wild Rats*

Observed	No. Parasites Found	Sex of Parasite	Position in Host
1/23/20	1	Young ♀	Urinary bladder, no ♂ in uterus
1/13/23	1	Young ♀	Urinary bladder, no ♂ in uterus
1/13/23	1	Young ♀	Urinary bladder near entrance of ureter; no ♂ in uterus
1/15/23	1	Young ♀	Ureter near bladder; no ♂ in uterus
1/29/23	1	Young ♀	Urinary bladder, no ♂ in uterus
3/ 3/23	3	Young ♀	Urinary bladder, male with stylet
3/ 3/23	1	Young ♀	Urinary bladder, not laying
3/ 3/23	1	Young ♀	Urinary bladder, not laying
3/ 5/23	1	Young ♀	Renal blood vessel, no ♂ in uterus
3/ 5/23	2	Young ♂	In renal tube in kidney
		Young ♀	In pelvis right kidney
3/ 5/23	2	Young ♀	Urinary bladder, not laying
3/15/23	1	Young ♀	Renal blood vessel, right kidney, no ♂ in uterus
3/17/23	1	Young ♀	Urinary bladder, not laying
3/24/23	3	Young ♀	Urinary bladder, not laying

males in their uteri occur in the urinary bladder. Either there was a deficiency of males in such cases, or males and females did not come together. Females after copulation become greatly distended posteriorly with developing eggs.

Apparently after a female has settled down as an egg-laying adult, she loses her stylet. The complete or partial envelopment of females in papillomas has been interpreted by the writer as indicating that the female has persisted in that situation for some time. The male may not stay permanently in the uterus of a female, for the writer has observed males in various stages of entering and leaving the female; also free males have been observed in the urine of freshly killed rats whereas females are always found attached by their anterior ends to the bladder epithelium. However, if the excised bladder is left for some time before examination, the females may extricate themselves.

SUMMARY

The Norway rat, *Epimys norvegicus,* in the vicinity of Urbana, Illinois, shows a 69.8 per cent. infection of *Trichosomoides crassicauda* (Bellingham) in the urinary tract.

Experiments indicate that infestation is brought about by the ingestion of eggs.

Uninfested stock associated in cages with infected rats contract the parasites.

Control rats not exposed to infection though kept in the same room with infested stock did not develop the parasite.

About three hours after the ingestion of eggs, the larvae hatch out in the stomach, pass into the blood stream and then by way of the portal system to the heart.

Newly hatched larvae possess a stylet, a prepuce-like fold at the anterior end, and an intestine. They may bore out into the body cavities or bronchi. Only the few lodged in the urinary tract find conditions favorable for their development.

Little growth takes place the first eight days in the rat.

Larvae require from three to six weeks to become egg-laying adults.

Experiments did not indicate that the larvae occur regularly in the lungs of the rat as believed by Yokogawa.

Deaths may occur in young rats from pulmonary troubles brought about indirectly by the wandering larvae.

Copulation takes place at any point in the urinary tract. The male enters the vagina of the female and commonly remains there but may wander out again.

The male retains the stylet and other larval charácteristics, whereas the female settles down in one situation and loses the stylet. A proliferation of epithelial cells often occurs at the point where the worm is imbedded.

The presence of a stylet suggests a possible relation with such partly free-living and partly parasitic nematodes as Dorylaimus and Tylenchus. Cells along the cloacal or rectal region of the male are strikingly similar to those found in Trichina and Dorylaimus.

LITERATURE CITED

Balfour, Andrew. 1922.—Observations on Wild Rats in England, with an Account of Their Ecto- and Endoparasites. Parasitol., 14: 282-298.

Bastian, H. C. 1864.—Monograph on the Anguillulidae, or Free Nematoids, Marine, Land and Freshwater; with Descriptions of 100 New Species. Trans. Linn. Soc., 25: 73-184.

Bellingham, O. 1840.—Catalogue of the Entozoa Indigenous to Ireland; with Observations. Mag. Nat. Hist., (43), n.s., 4: 343-351.

1844.—Catalogue of the Irish Entozoa, with Observations. An. and Mag. Nat. Hist., 14: 471-480.

Brumpt, E. 1921.—Mode de penetration de Nematodes dans l'organisms des Mammiferes, histiotropisme et histiodiagnostic. Compt. rend. soc. biol., 85 : 203-206.

Bütschli, O. 1872.—Beobachtungen über mehrere Parasiten. Arch. Naturg., 1 : 236-241.

1873.—Beiträge zur Kenntniss der freilebenden Nematoden. Nova Acta. Leop. Carol. Akad. Naturfor., 36 : 3-144.

Diesing, C. M. 1851.—Systema Helminthum.

Dujardin, F. 1845.—Histoire naturelle des helminthes ou vers Intestinaux. Paris.

Eberth, C. J. 1863.—Untersuchungen über Nematoden. Leipzig.

Fuchs, G. 1915.—Die Naturgeschichte der Nematoden und einiger anderer Parasiten 1. des *Ips typographus* L. 2. des *Hylobius abietis* L. Zool. Jahrb., Syst., 38 : 109-222.

Fülleborn, F. 1914.—Untersuchungen über den Infektionsweg bei Strongyloides und Ankylostomum und die Biologie dieser Parasiten. Arch. Schiffs-Trop. Hyg., 18 : 26-80.

1920.—Ueber die Anpassung der Nematoden an den Parasitismus und den Infektionsweg bei Askaris und anderen Fadenwürmern des Menschen. Arch. Schiffs-Trop. Hyg., 24 : 340-347.

1921.—Ueber Askaridenlarven im Gehirn. Arch. Schiffs-Trop. Hyg., 25 : 62-63.

Goodey, T. 1922.—Observations on the Ensheathed Larvae of Some Parasitic Nematodes. Ann. Applied Biol., 9 : 33-48.

Hall, M. C. 1916.—Nematode Parasites of Mammals of the Orders Rodentia, Lagomorphia, and Hyracoidea. Proc. U. S. Nat. Mus., 50 : 1-258.

Jägerskiöld, L. A. 1901.—Weitere Beiträge zur Kenntnis der Nematoden. Kongl. sv. Vet. Akad. Handl., 35 : 1-80.

Johnston, T. H. 1918.—Notes on Certain Entozoa of Rats and Mice, Together with a Catalogue of the Internal Parasites Recorded as Occurring in Rodents in Australia. Proc. Royal Soc. Queensland, 30 : 53-78.

Leuckart, R. 1867.—Ueber Trichosoma crassicaudatum aus der Harnblase der Wanderratte. Cor. Blatt. zoolog.-mineral. Verein, Regensburg, 21 : 148.

1867a.—Ueber Trichosoma crassicaudatum aus der Harnblase der Wanderratte. Tageblatt d. 41. Versamml. Deutsch. Natur. u. Aerzte, 5 : 49-64.

Linstow, O. von. 1874.—Beobachtungen an Trichodes crassicauda Bell. Arch. Naturg., 40 : 271-286.

1882.—Helminthologische Studien. Arch. Naturg., 48 : 1-25.

1897.—Zur Systematik der Nematoden nebst Beschreibung neuer Arten. Arch. mikr. Anat., 49 : 608-622.

Looss, A. 1892.—Schmarotzertum in der Tierwelt. Zool. Vorträge, 10 : 1-180.

1905.—The Anatomy and Life History of Agchylostoma duodenale Dub. Rec. Egypt. Govt. Sch. Med., 3 : 7-147.

1911.—The Anatomy and Life History of Agchylostoma duodenale Dub. Rec. Egypt. Govt. Sch. Med., 4 : 167-607.

Löwenstein, S. 1910.—Epithelwucherungen und Papillombildungen der Rattenblasse, verursacht durch ein Trichosoma (Tr. crassicauda?). Beitr. klin. Chir., 69 : 533-546.

1911.—Trichodes crassicauda specifica als Erreger von Papillomen der Blase und Niere. Verhandl. Gesell. Deutsch. Naturf. Artzte, 83 : 162-166.

1911a.—Trichodes crassicauda specifica, eine Causa directa in der Aetiologie der Tumoren. Beitr. klin. Chir., 76 : 750-769.

1912.—Ueber Trichodes crassicauda specifica, eine Causa directa in der Aetiologie der Tumoren. Münch. med. Wochenschr., 59 : 324.

1913.—Ueber durch Nematoden hervorgerufene Geschwulst-bildungen bei der Ratte. Berl. klin. Wchnschr., 50 : 761-763.

Railliet, A. 1895.—Traité de zoologie medicale et agricole. 2 ed. Paris.

Ransom, B. H. 1922.—Some Recent Additions to the Knowledge of Ascariasis. Jour. Amer. Med. Assn., 79 : 1094-1097.

Ransom, B. H., and Foster, W. D. 1920.—Observations on the Life History of Ascaris Lumbricoides. U. S. Dept. Agr. Bull., 817: 1-47.

Ransome, B. H., and Cram, E. B. 1921.—The Course of Migration of Ascaris Larvae. Amer. Jour. Trop. Med., 1: 129-156.

Rauther, M. 1918.—Mitteilungen zur Nematodenkunde. II. Zur Anatomie der Trichotracheliden. Zool. Jahrb., Anat., 40-465-499.

Rayer, P. 1843.—Sur des Trichosomes observes dans la vessie du surmulot (Mus decumanus) et dans la vessie du ranard commun (Canis vulpes). Arch. med. comp., 2: 180-182.

Romanovitch, M. 1912.—Recherches sur la Trichinose. Ann. Inst. Pasteur, 26: 352-370.

Saul, E. 1914.—Untersuchungen zur Aetiologie und Biologie der Tumoren. Helminthen und Protozoen. Centralbl. Bakt. Parasit. Abt. 1, Orig., 75: 295-211.

Stewart, F. H. 1921.—On the Life-History of Ascaris lumbricoides Lin. Parasitol., 13: 37-47.

Stossich, M. 1890.—Il Genere Trichosoma Rudolphi. Triest.

1898.—Saggio di une Fauna Elmintologica die Trieste e Provincie Contermini.

Veglia, F. 1915.—The Anatomy and Life-History of Haemonchus contortus. Rept. Dir. Vet. Research, South Africa, 34: 347-500.

Walter, H.: 1866.—Helminthologische Studien. Ber. Offenbacher Ver. Naturk. 7: 51-79.

Ward, H. B. 1917.—On the Structure and Classification of North American Parasitic Worms. Jour. Parasitol., 4: 1-12.

Yokogawa, S. 1921.—On the Migratory Course of Trichosomoides crassicauda (Bellingham) in the Body of the Final Host. Jour. Parasit., 7: 80-84.

Yoshida, S. 1919.—On the Migrating Course of Ascarid Larvae in the Body of the Host. Jour. Parasit., 6: 19-27.

Explanation of Plates

All figures represent *Trichosomoides crassicauda* (Bellingham).

All drawings are made witth the camera lucida and the projected scale indicating magnification in each instance has the value of 10μ unless otherwise stated. All sections were cut 3μ thick.

Abbreviations: *c*, cuticular ridges; *cg*, cuticular glands; *g*, esophageal glands; *m*, metaplasm granule; *rc*, rectal cells; *t*, tonoplast.

Explanation of Plate XIV

Fig. 1.—Larva just forced from shell by pressure, showing retracted stylet, prepuce, and hyaline granules; metabolism products and parts of vitelline membrane extruding from opercular cap. Scale 50μ long.

Fig. 2.—Living larva from abdominal cavity of rat Exp. 12.

Fig. 3.—Anterior end of contracted larva just escaped from egg. Cross-section (*3a*) through anterior end, (*3b*) through intestine, (*3c*) near extreme posterior end, (*3d*) longitudinal section through mid body region.

Fig. 4.—Living larva from lung of rat Exp. *3*.

Fig. 5.—Eggshell from stomach of rat Exp. *32*; scale 50μ long. (*5a*) Transverse section of shell showing fertilization membrane and rugose outer layer.

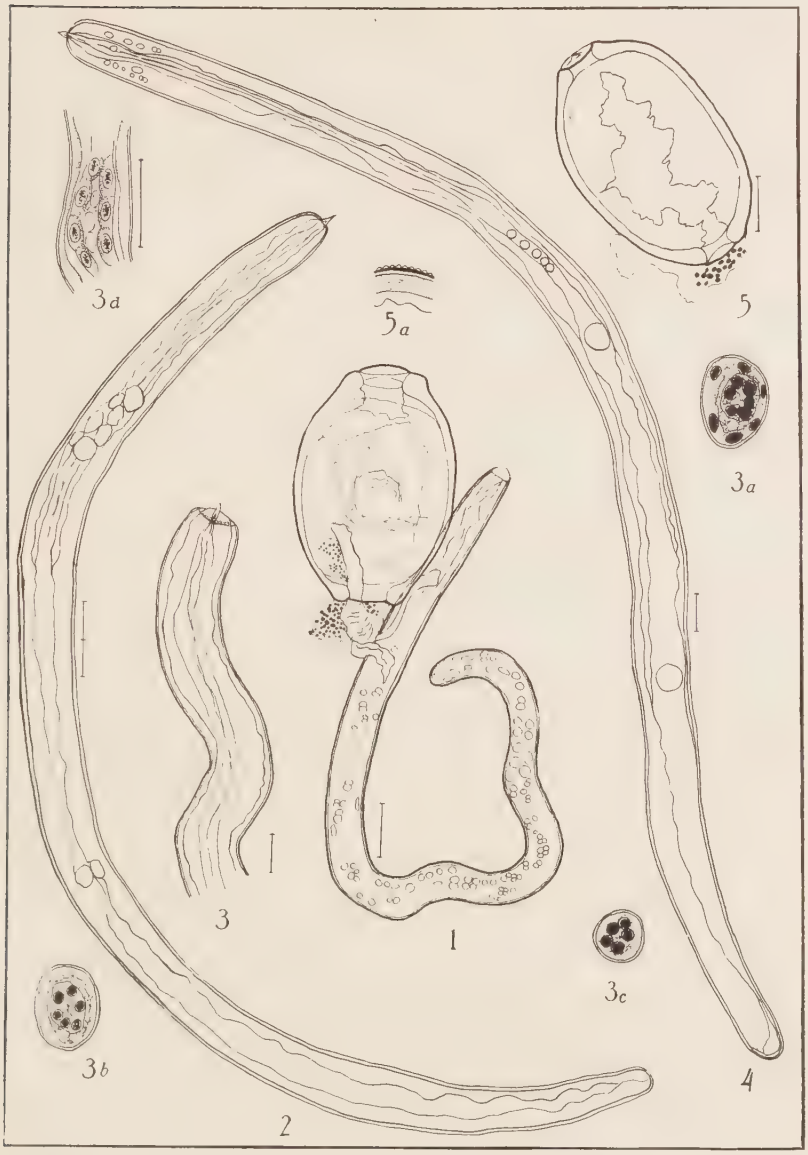


PLATE XIV

Explanation of Plate XV

Fig. 6.—Section through female showing nerve ring and tripartite lumen of esophagus.

Fig. 7.—Section more posterior showing beginning esophageal cells.

Fig. 8.—Large nucleated esophageal cell of female.

Fig. 9.—Section through nonnucleated part of a similar cell.

Fig. 10.—Esophgaeal cell showing metaplasm granule.

Fig. 11.—Section near posterior end of female esophagus.

Fig. 12.—Section further posteriad than figure 11, showing cuticular ridges and tonoplasts.

Figs. 13-17.—Corresponding sections through similar regions in the male.

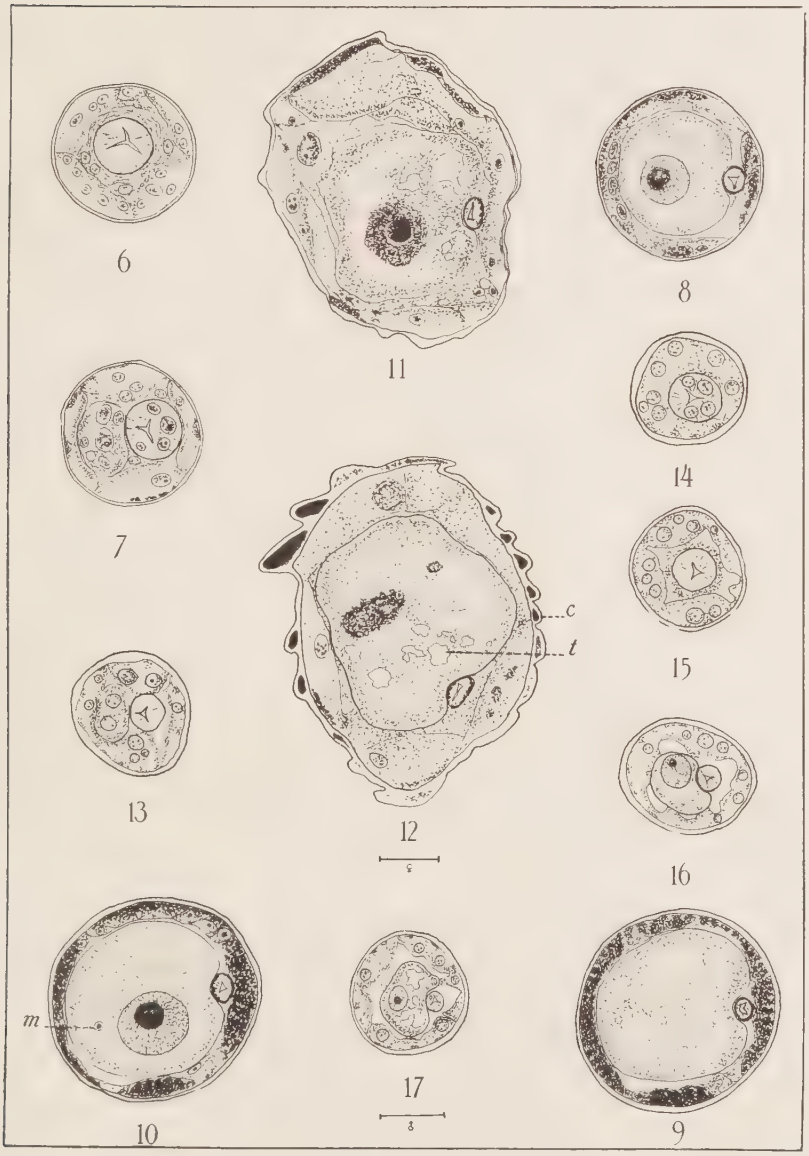


PLATE XV

Explanation of Plate XVI

Fig. 18.—Anterior end of living male from ureter of rat Exp. 4, with stylet protruding.

Figs. 19-20.—Esophagus and esophageal cells.

Figs. 21, 22 and 23.—Intestine and testis.

Fig. 24.—Cloacal region with large hyaline cells near rectum.

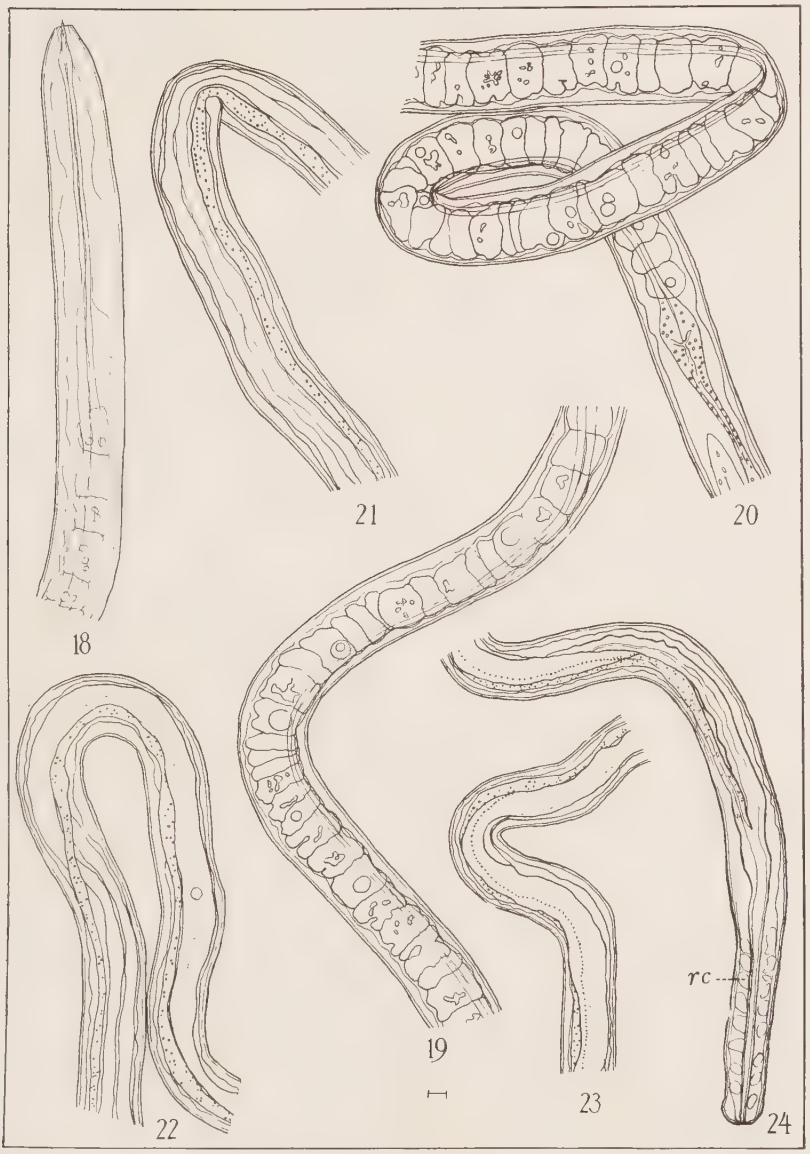


PLATE XVI

EXPLANATION OF PLATE XVII

Fig. 25.—Posterior end of adult male under pressure.

Fig. 26.—Anterior end of young female from lumen of left ureter of rat showing stylet brought out under pressure.

Fig. 27.—Anterior end of living male, stylet retracted.

Fig. 28.—Newly hatched larva with portion of vitelline membrane and metabolism products adhering, stained to show nuclei.

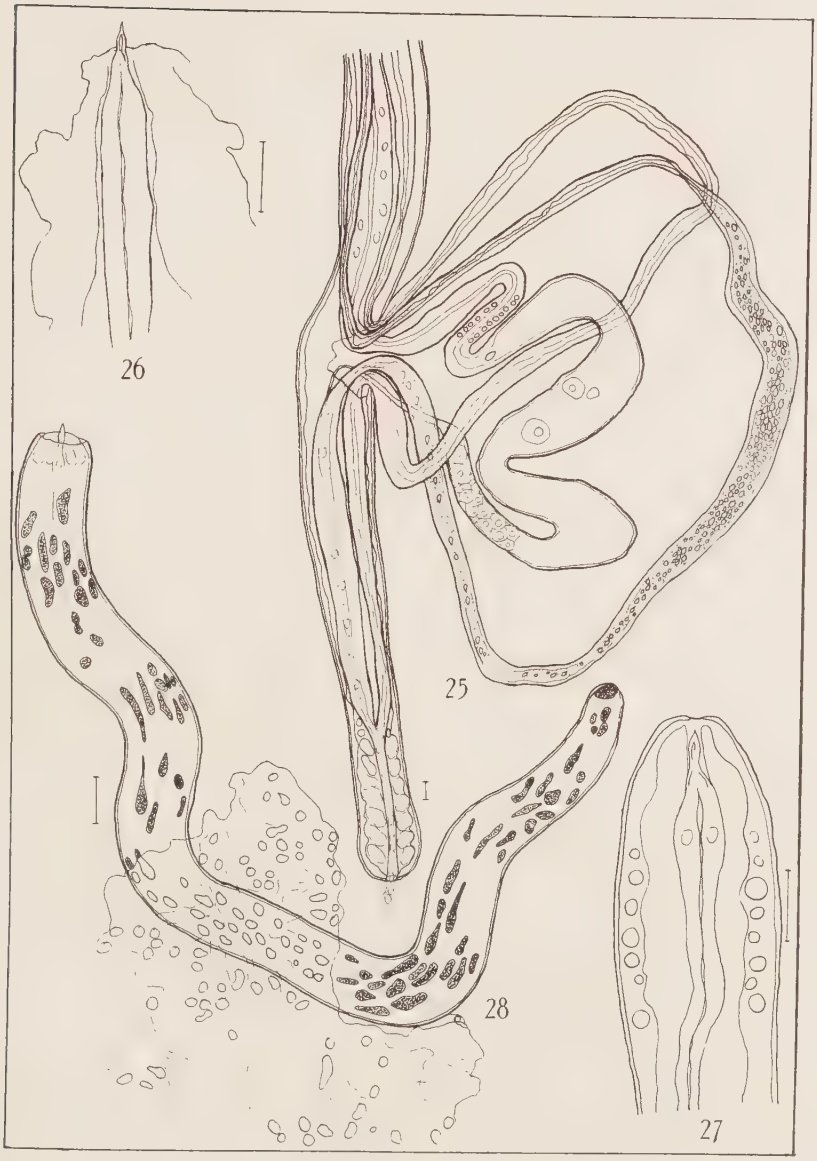


PLATE XVII

Explanation of Plate XVIII

Fig. 29.—Tangential section through vagina, intestine, and esophagus, showing cuticular glands and cuticular ridges of adult female.

Fig. 30.—Longitudinal section through esophagus near juncture with intestine showing portion of esophageal gland of female.

Fig. 31.—Longitudinal section through same region as figure 30, showing large esophageal cells.

Fig. 32.—Longitudinal section through female showing valves separating esophagus and intestine.

Fig. 33.—Toto mount of anterior end of male stained, showing stylet, musculature, and prepuce.

Fig. 34.—Vaginal region of living young female from kidney pelvis.

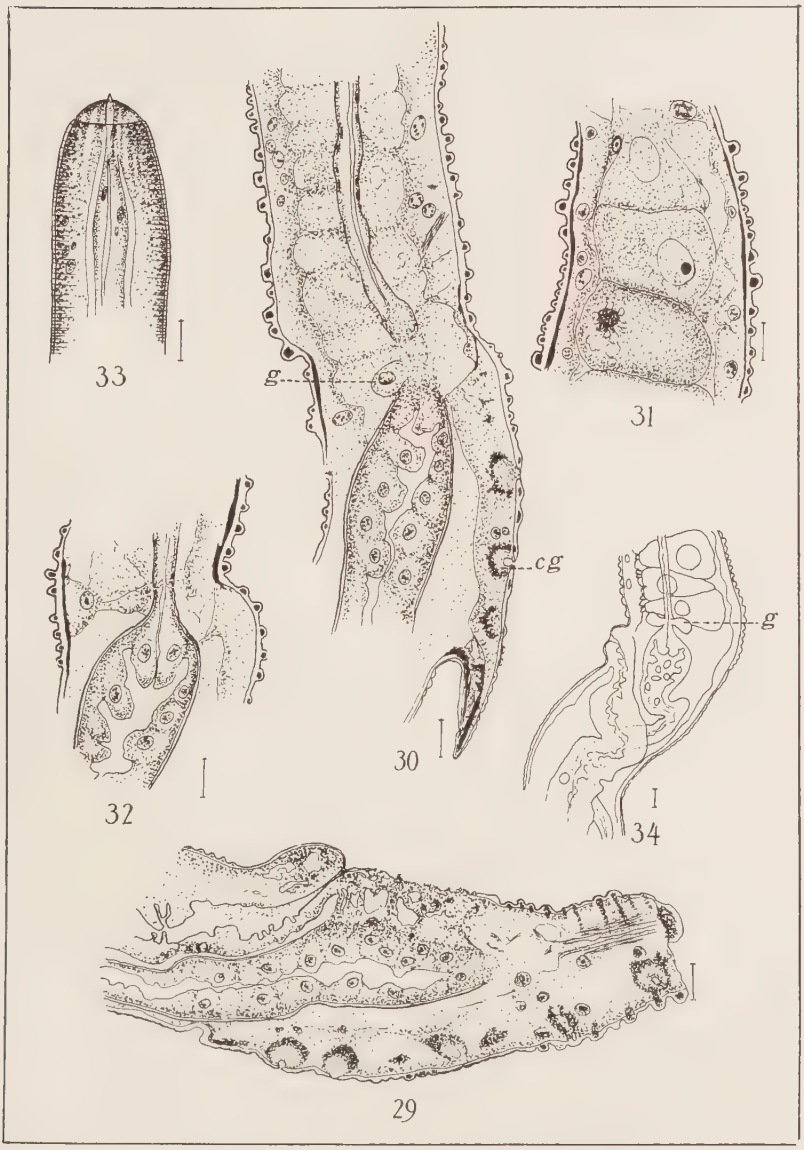


PLATE XVIII

NOTES ON COCCIDIAL OÖCYSTS FROM DOMESTIC ANIMALS IN CALIFORNIA

NELSON C. DAVIS AND W. W. REICH

From the Department of Bacteriology and Experimental Pathology
University of California, Berkeley

It seems worth while to report the finding of several species of Coccidia in California. Our attention was drawn to coccidiosis by its prevalence among rabbits in the bacteriological laboratories of the University of California at Berkeley. Many valuable animals were lost and numerous experiments were interrupted. We then examined fecal specimens from a considerable number of apparently healthy animals and, from the results, estimated that at least 75 per cent. of the adults were carriers of *Eimeria stiedae*. Rudovsky (1921) has called attention to the presence of *E. falciformis* and *E. stiedae* in the rats found infesting rabbit hutches. He suggests that the rat may act as a carrier of coccidiosis and constitutes a natural reservoir. Our own observations on a limited number of both wild rats and laboratory mice have been negative.

In acute cases oöcysts are readily demonstrable in direct fecal smears, but as a routine we have developed a centrifuge method of concentration, similar to that frequently employed in helminth examinations. The fecal specimens are mixed with water and allowed to soften, if dry, then are mashed and strained through gauze to remove the coarser particles; the filtrate is centrifuged at a speed of between 1200 and 1500 revolutions per minute for ten to fifteen seconds, the filtrate is poured off and the sediment is washed and re-whirled at the same rate from one to three times, depending upon the amount of fine, light material to be eliminated. The final sediment may be mixed with water, spread upon a slide, and examined without using a coverslip; in most cases the lower magnifications obtainable with an ordinary compound microscope are adequate for recognition of oöcysts.

Until a comparatively recent date it has been the custom of investigators to refer all coccidial infections, or at least all those occurring in mammals, to the common rabbit form, *Eimeria stiedae* (*Coccidium oviforme, C. cuniculi, C. perforans*). Since the excellent articles of Wenyon (1915-1916), Dobell (1919) and others have appeared, there is no excuse for confusing the human parasites with those of the rabbit. There is also a tendency to separate various animal species from *E. stiedae*. Nothwithstanding the fact that species of coccidia seem to be limited in the number of their hosts, it is highly desirable to know the extent of the cross-infections which may, and do, occur; it is possible that some of the four or five species now known to parasitize man may have an animal reservoir in nature. The infrequency of coccidial

infections in man, especially with species of Eimeria, suggests the possibility that human beings are chance or accidental hosts, or at least that they share their infestation with others.

Since the result of our concentration method proved successful in demonstrating oöcysts in rabbit carriers, we extended our search to include other animals. Unfortunately the laboratory chickens and pigeons were not all isolated in separate pens. However, all feces examined from these birds proved to contain many oöcysts of *Eimeria avium*. Fecal material from twenty dogs has been examined. Of these animals two showed oöcysts of *Isospora bigemina;* only one cyst was found in one of these cases, the other dog was moderately infested. Material from several cats was examined; a single specimen from one isolated animal was negative. Of the other four samples, representing collections from about fifteen, two had oöcysts of *Isospora bigemina.* We have examined the kidneys of a number of guinea pigs, especially for Klossiella, but with negative results. Collective fecal samples from cages of laboratory rats have yielded many ova of *Hymenolepis diminuta,* but no coccidia. A few white mice have also been negative.

Several trips to the slaughter houses and packing houses of West Oakland have proven fruitful. According to the employees at these places, the animals come mainly from California, with lesser numbers from Oregon, Nevada, and Arizona. No attempt has been made to determine the exact point of origin of those animals whose intestinal contents have been examined. We obtained specimens of semi-fluid, large intestinal contents from six sheep, two specimens secured on one trip, and the other four three weeks later. All six contained moderate numbers of Eimeria oöcysts. The droppings from four laboratory sheep have furnished no cysts. Contents of cecum and colon from nine hogs were examined, three specimens in January, the other six in March. The first three were negative. Of the last six specimens, five showed Eimeria oöcysts. Large intestinal contents have been collected from four slaughtered cattle, two specimens upon each of two occasions at an interval of about eight weeks. The last two harbored an Eimeria.

It has been our experience that oöcysts containing developed sporozoites, or even sporocysts, are rare in fresh samples of feces. Developmental forms may be obtained in a few days by keeping the fecal material moist at room temperature. It is advisable to spread out the feces in an open Petri dish; development may be retarded in samples kept in test tubes or in deep bottles, probably from insufficient oxygen. We plated some specimens on agar, but it is doubtful whether there was any advantage in this procedure. We have suspected that there may be occasionally further development after preservation in 10 per cent. formalin (4 per cent. formaldehyde) solution.

Certain features common to all species of Eimeria which we have encountered may be briefly reviewed to prevent repetition. Many young

forms are frequently found in which the protoplasm is granular, and variation in size of granules is apparently individual rather than specific. More frequent and probably later, stages show the protoplasm rounded up, and more or less centrally located, although the mass may be in contact with the wall at some place, or may be nearer one end than the other (more often toward the micropylar extremity). Within the globular mass of protoplasm it is usually possible to see the nucleus (later nuclei). By rapid focusing the protoplasm is seen to include four rounded masses, the sporoblasts; these latter elongate and develop definite walls, the sporocyst capsules. Within each sporocyst two falciform sporozoites develop. It is often difficult to distinguish the outlines of the sporozoites in fresh material; there may be slight individual, as well as specific, variation in the exact position of the sporozoites within their sporocysts. The broad or head end, of the sporozoites usually appears more dense and homogeneous than the body and tail. Varying with the species the nucleus is centrally located, or nearer the broad end; each nucleus appears as a halo, usually with a discernible central, black dot, the nucleolus. In every species studied by us there appeared to be a protoplasmic residual body, within each sporocyst (after the formation of sporozoites) which might or might not be condensed into a definitely outlined mass, possibly dependent upon the stage observed.

Eimeria stiedae (Lindemann 1895)

It seems scarcely necessary to discuss or to describe this common parasite of the rabbit; it is described fully in all standard works on parasitology or protozoology. The oöcysts are refractive, translucent, and colored a delicate salmon tint which in larger, older cysts may become darker almost a light brown. The shape of the oöcysts is ellipsoidal or oval, with a definitely differentiated micropylar end (Figs. 1, 2). The micropylar extremity is somewhat flattened, with thickened wall at the corners, with often a thickening or bulging outward or depression at the pole. The sporocysts are fusiform and usually demonstrably thicker at one end than at the other. There is a definite protoplasmic residue after sporocyst formation.

Measurements of fully grown cysts in our material have run from 36 to 42μ in length by 23 to 30μ in width. The literature gives a somewhat wider range than these figures indicate:

Doflein, 20-50 by 20-39μ; Haughwout, 33-49 by 15-28μ; Chaine, 30-50 by 20-30μ.

Eimeria avium (Silvestrini and Rivolta 1873)

This is another common parasite of wide distribution. The life history has been worked out by Fantham upon material from grouse. It causes "white diarrhea" of fowls, and also produces fatalities in

turkeys, geese, ducks, pea-fowl, pigeons, pheasants, grouse, and other aviary species. The oöcysts observed by us have been spherical or subspherical, very thick walled, not colored, without visible micropyle, and measured 18 to 24μ by 16 to 21μ with an average of 21.2μ by 18.6μ in size (Figs. 3, 4). The sporocysts are almost peg-shaped; there is no protoplasmic residue within the cyst after sporocyst formation. The sporozoites appear to be rather small and comma-shaped.

The type described by Fantham from the grouse usually had ovoid oöcysts, occasionally pyriform, rarely subspherical. The measurements were 25 to 35μ in length by 14 to 20μ in width; the subspherical forms measured 18 to 20μ in diameter. Fantham says that there is a minute amount of resdiual cytoplasm in the fully developed oöcysts, but he does not show it in his figures. Morse found circular oöcysts, sometimes slightly oval forms; the cysts were 12 to 25μ in diameter. Jowitt gives the measurements as 15 to 23μ by 15 to 25μ.

Eimeria from sheep

The oöcysts of this species are ellipsoidal or ovoidal, and in size vary from 24 to 40μ in length by 17 to 25μ in width. The color is a delicate salmon tint in all stages observed. Sporocysts are elongated, bluntly rounded at one pole and slightly tapering toward the other. There is no protoplasmic residue after formation of sporocysts. The most striking characteristic about the oöcysts is the bulging cap or nodule at the micropylar end. This is observable in every cyst (Figs. 5-8). *Eimeria faurei* described from sheep by Moussu and Marotel agrees very well in many respects with this form, but the very distinctive micropylar cap is neither figured nor described in the original article. The measurement as given for *E. faurei* are 30 to 40μ by 18 to 26μ with larger forms 42 by 30μ and smaller subspherical ones 17μ in diameter.

Nocard has described small coccidia (probably Klossiella) from sheep, 10 to 12μ by 7 to 9μ. Curtice found coccidia in sheep, and the organism was described by Stiles from sections of the intestinal lesions. Cysts found in these sections measured 18 to 21μ by 15 to 16μ. They were thought to be similar, perhaps identical, to *Coccidium performans* (*Eimeria stiedae*). Mazzanti described coccidiosis in lambs; the oöcysts measured 30 to 50μ by 14 to 26μ; the parasites were thought to have the same history as those of the rabbit, and the two forms were considered identical. Unfortunately this last paper has not been available to us. Stevenson has reported coccidiosis in goats in Africa, but he does not give measurements or adequate descriptions of the oöcysts.

Eimeria zurni from cattle

The form found by us in large intestinal contents of cattle is faintly colored, resembling in this respect the sheep Eimeria. The shape of the oöcysts is ovoid, usually with a change of contour, sometimes a definite

flattening at the micropylar extremity. The wall is thick at the bluntly rounded end but gradually thins toward the other extremity; if a flattening is present, the corners are rounded and not thickened. The sporocysts taper distinctly toward one end; there is no residual protoplasm after sporozoite formation. Oöcysts measurements vary from 24 to 34μ by 17 to 21μ, with perhaps a preponderance of the larger forms. Figures 17 and 18 give some idea of the range in size that we encountered.

Eimeria zurni is named for Zurn who first found coccidia in calves in 1878. It causes "red diarrhea" in cattle. The disease is apparently prevalent in Central Europe, and has been described from various other parts of the world. Montgomery has reported cattle coccidia in Africa. He describes the oöcysts as clear and transparent, with finely granular protoplasm frequently aggregated at the pole opposite the micropyle; in size they were 14 to 20μ by 12 to 18μ. Balfour also reports coccidiosis among African cattle, but gives very little morphological data. Jowitt, writing from Cape Town, discusses *Coccidium bovis* found in calves; the oöcysts are usually round, often 16 to 17μ in diameter. Subspherical forms measure 16 by 14.4μ, the largest cysts are 27.2 by 20.8μ, and the smallest 14.4 by 12.8μ. Schultz has studied coccidiosis among cattle and carabaos in the Philippines. The form found was thought to be *Eimeria stiedae*. Linton was among the first to report "red diarrhea" in England, but he gives little description of his material.

In America, Reichel reported cases in 1910 in cattle, horses, and a goat which he considered coccidiosis; the cysts were only 2.5 by 5μ in size. Probably the first proven cases in this country were presented by Jervis in 1914. Several yearling cattle and one cow were killed by the disease. Oöcysts were round to subspherical, 16 to 18μ and a few up to 20μ in diameter. Smith and Graybill report cases of coccidiosis in calves, in which two forms of oöcysts were found, thought to belong to separate species. The first form was small and usually elliptical, occasionally ovoid; the oöcysts wall was uniform in thickness, the average size was 18.6 by 14.8μ; no residuum was present in the sporocysts. The second form was larger and ovoid; the cyst wall was thinner at the narrow end, but no micropyle was present. The average size was 29.9 by 19.9μ, with a residuum in the sporocyst.

Eimeria specimens from hogs

This form was found in the intestinal contents of five slaughtered hogs. The oöcysts closely resemble those recognized in cattle; in fact it is not possible to give any points of difference of a constant nature. The color is about the same as the preceding, although older cysts are

brownish. The walls may show fine, irregular cross striations; these are more rarely found in the cattle form, described above. The thickness of the walls is usually fairly uniform, and the micropylar end is seldom differentiated, but when polarization is exhibited it is shown as a slight flattening and thinning as in the previous case. The shape may be ovoidal, but many ellipsoidal forms (Fig. 15) and some subspherical forms (Fig. 16) are found. The size corresponds to that of the smaller cattle Eimeria, 19 to 26μ by 16 to 23μ. Sporocysts tend to be a little longer than in the other form (compare Figs. 16 *m* and 18 *m*); there is usually no residual protoplasm after the formation of sporocysts, although in one case a small, homogeneous, residual body was observed. We have been able to find no literature upon coccidia in swine.

Isospora bigemina (Stiles 1891)

In fresh material from cats and dogs *Isospora bigemina* appears in various stages of development; there may be a single rounded mass of protoplasm, two sporoblasts may be present, or two slightly ovoidal sporocysts showing numerous variations of internal structure. Full sporozoite formation (four to each of the two sporocysts) is comparatively uncommon in fresh feces.

The oöcyst wall is thin and uncolored. The shape is spherical, slightly ovodial, or slightly ellipsoidal. The size varies considerably with the race; the forms which we found in dog and cat feces are small, usually lying between 25 and 30μ in length by 20 to 25μ in width (Figs. 9, 10). Some cat material obtained from Dr. E. L. Walker contains oöcysts 35 to 47μ by 27 to 40μ, with an average of 41 by 33μ. There is no protoplasmic residue within the oöcyst after formation of sporocysts, but each sporocyst contains a protoplasmic remainder after sporozoites are formed; this "rest" is granular, but may be condensed into a spheroid; the granules vary immensely in size among different cysts. The sporozoites observed by us seldom approach a falciform shape; the ends are rounded or slightly pointed, but equally so; one side often appears flat, sometimes slightly in-curved; the nucleus is seen as a halo usually in midposition.

Figures 11 to 14 represent small cysts found in the stools of a dog which also showed oöcysts of *Isospora bigemina* (Figs, 9, 10 are from this case.) The size of these cysts (averaging 17 by 11μ) and their internal structure suggest very strongly that they are liberated sporocysts from the *I. bigemina* oöcysts; the walls are, however, rather too thick for such an interpretation. Some of these in fresh material apparently had only two bodies within them (not the full four sporozoites); no *bigemina* oöcysts were seen at first examination which showed any

division within the sporocysts. No free sporocysts were found in the other cases of *I. bigemina* infection, not even in the heavily parasitized case whose feces were obtained from Dr. Walker.

Isospora bigemina has been described from the cat, the dog, the French pole-cat, and from Swift foxes. Therefore, we have at least four varieties: *I. bigemina cati, I. bigemina canis, I. bigemina putori,* and *I. bigemina canivelocis.* In the original article of Stiles the size of *I. bigemina* "single" cysts is given as 13.5 to 15.9μ by 7.9 to 9.9μ, that of " twin" cysts as 10.6 by 9.3 to 10.3μ. He mentioned cases in which only one of the twin sporocysts developed. Haughwout (1918) gives a variation in size of 29 to 38μ by 22 to 29μ. Hall mentions finding two strains, a larger, more common strain 36 to 40μ by 28 to 32μ, and a smaller strain in one animal 20 by 18μ. Mature oöcysts have been reported from foxes; they measured 25 to 38μ by 25 to 30μ.

DISCUSSION

Without a full life history worked out for each form, and extensive experimentation to determine the range of variation, it is a question as to how far size and shape may be taken as specific characteristics. We now speak of "races," or "strains" of *E. avium, E. zurni, I. bigemina,* etc., but it is not beyond the realms of possibility that some of these variable species may be split up some day. Fantham has suggested that small oöcysts occur in heavy infections because they neither have food nor room to grow into large forms. This is not always the case, however; all the *E. avium cysts* which we encountered were relatively small and subspherical, but none of the fowls or pigeons were clinically ill, and the numbers of cysts indicated only moderate infections. Furthermore, in the *Isospora bigemina* infections, which were light or moderate, only small oöcysts were found, while in the material given us by Dr. Walker, which showed very great numbers of cysts, a large "strain" was present. Color, also, is a variable characteristic, depending upon the age of the oöcyst, and possibly upon the content of bile and other pigments in the intestinal tract of the host.

We consider that the structure of the micropylar end of the oöcyst is a valuable distinguishing characteristic; thus, we should not hesitate to separate the Eimeria found by us in sheep, from the type species in sheep, *Eimeria faurei,* upon the presence of the large, nodular, micropylar cap alone, providing the original description and drawings are accurate. Other valuable points, which seem to be constant, are the presence or absence of protoplasmic residues, either within the oöcyst after the differentiation or sporocysts, or within the sporocysts after formation of the sporozoites. The presence of a large globular mass of residual protoplasm outside the sporocysts in *Eimeria stiedae,* should

serve to separate it from some other forms with which it has been confused.

After the study of oöcysts in the cases of swine coccidiosis, we are left in considerable doubt as to their significance. There are some points of difference between this form and that found in the cattle examined but there is an overlapping borderland of similar forms. The scavenger habits of the hog make it peculiarly liable to chance infections; it is quite possible that swine pastured with cattle harboring *Eimeria zurni* might acquire an infection. That coccidiosis of swine is not of pathogenic nor of economic importance is suggested by that fact that veterinarians are not cognizant of its existence. Dr. Walker told us verbally that he had seen coccidia in hogs, but the literature on the subject is scanty or lacking. For the present we shall consider the small cysts found in one dog as being free sporocysts of *Isospora bigemina*.

The material secured at the West Oakland packing houses from cattle, sheep, and hogs was all from normal animals, or at least from those that were considered suitable for slaughter and human consumption. Furthermore, the dogs, cats, chickens, pigeons, and many of the rabbits examined were apparently healthy, and yet in a considerable percentage coccidial infections were found. This indicates the prevalence of carrier states among our domestic animals; these carriers are obviously a source of danger to young animals and to older nonimmunes. Coccidial infections are recognized diseased entities among all the animals discussed, except hogs; the ravages are especially marked among the young of the various species. That these diseases are present here in the West, perhaps in a mild or latent form, should awaken some desire for control measures to prevent their spread. The recent work of Kuczyuski, Patterson, and others on complement fixation reactions for the diagnosis of Eimeria infections may form the basis for a useful test, especially in the case of young carriers. In the absence of accurate data regarding the rôle played by coccidia as disease incitants among animals in general it would seem worth while to encourage studies in this direction.

From the point of view of scientific parasitology we wish to emphasize the ready possibility of securing slaughterhouse material to study the schizogonic and early sporogonic phases of these parasites. It would be quite easy to obtain the whole intestinal tract from animals in order that sections might be made at various levels for histopathological studies. We only regret that we have had no time for tissue work.

SUMMARY

1. Coccidial infections are reported in rabbits, chickens, pigeons, dogs, cats, sheep, cattle, and swine. Previous data regarding these infections in California are not numerous.

2. An Eimeria has been found in sheep. It is believed that this species has not been previously reported.

3. The Eimeria in hogs may be a separate species, or may be a modified strain of the form found in cattle. We have found no previous report of coccidia in swine.

We wish to express our thanks to Dr. E. L. Walker for the material obtained from him, also to the various university departmental laboratories for allowing us the liberty of their animal rooms. We are also under obligations to the several West Oakland packing companies for the specimens obtained from them, and to the municipal meat inspectors for their unfailing courtesy and generous help.

References Cited

Dobell, C. 1919.—A Revision of the Coccidia Parasitic in Man. Parasitology, 11: 147.

Dobell, C., and O'Connor, F. W. 1921.—The Intestinal Protozoa of Man. Wm. Wood & Co., New York.

Doflein, F. 1916.—Lehrbuch der Protozoenkunde, Jena.

Haughwout, F. G. 1918.—Infections with Coccidum and Isospora, etc. Philippine Jour. Sci., Sec. B, 13: 79.

Moussu, G., et Marotel, G. 1902.—La Coccidiose de Mouton, etc. Arch. de Parasitologie, 6: 82.

Rudovsky, F. 1921.—Die Kokzidiose der Wanderratte (Mus decumanus Pall), etc. Cent. f. Bakt., 1 Abt. Orig. 87: 427.

Wenyon, C. M. 1915.—Observations on the Common Intestinal Protozoa of Man. Lancet, Nov. 27, 1173. 1915*a*.—The Development of the Human Coccidium: An Addendum. Lancet, Dec. 11, 1926. 1915*b*.—Another Human Coccidium from the Mediterranean War Area. Lancet, Dec. 25, 1404. 1916.—The Protozoological Findings in Five Hundred Fifty-Six Cases of Intestinal Disorder, etc. Jour. Roy. Army Med. Corps, 26: 445.

Addendum

Since this article went to press, we have had opportunity to review a recent article in which swine coccidiosis is described (Sheater, A. L., 1923—The Detection of Intestinal Protozoa and Mange Parasites by a Flotation Technique. Jour. Comp. Path. & Therapeutics, 36: 266).

Explanation of Plate XIX

Fig. 1.—*Eimeria stiedae.* Oöcyst with undivided protoplasmic mass.

Fig. 2.—*Eimeria stiedae.* Oöcyst with residual protoplasm, and four sporocysts each with two sporozoites.

Fig. 3.—*Eimeria avium* from pigeon. Oöcyst with undivided protoplasm.

Fig. 4.—*Eimeria avium* from pigeon. Oöcyst showing sporocysts and sporozoites.

Figs. 5 and 6.—*Eimeria* specimen from sheep, showing variation in size of oöcysts.

Figs. 7 and 8.—*Eimeria* specimen from sheep—developmental forms with sporocysts and sporozoite formation.

Fig. 9.—*Isospora bigemia* from dog. Two sporocysts.

Fig. 10.—*Isospora bigemina* from dog. Two sporocysts, each containing four sporozoites and a protoplasmic rest.

Figs. 11 and 14.—Small cysts from dog, probably liberated sporocysts of *Isospora bigemina.*

Fig. 15.—*Eimeria* specimen from hog.

Fig. 16.—*Eimeria* specimen from hog. Subspherical oöcyst with four sporocysts.

Fig. 17.—*Eimeria zürni* (?) from cattle feces.

Fig. 18.—*Eimeria zürni* (?), showing four sporocysts, each with two sporozoites in process of differentiation.

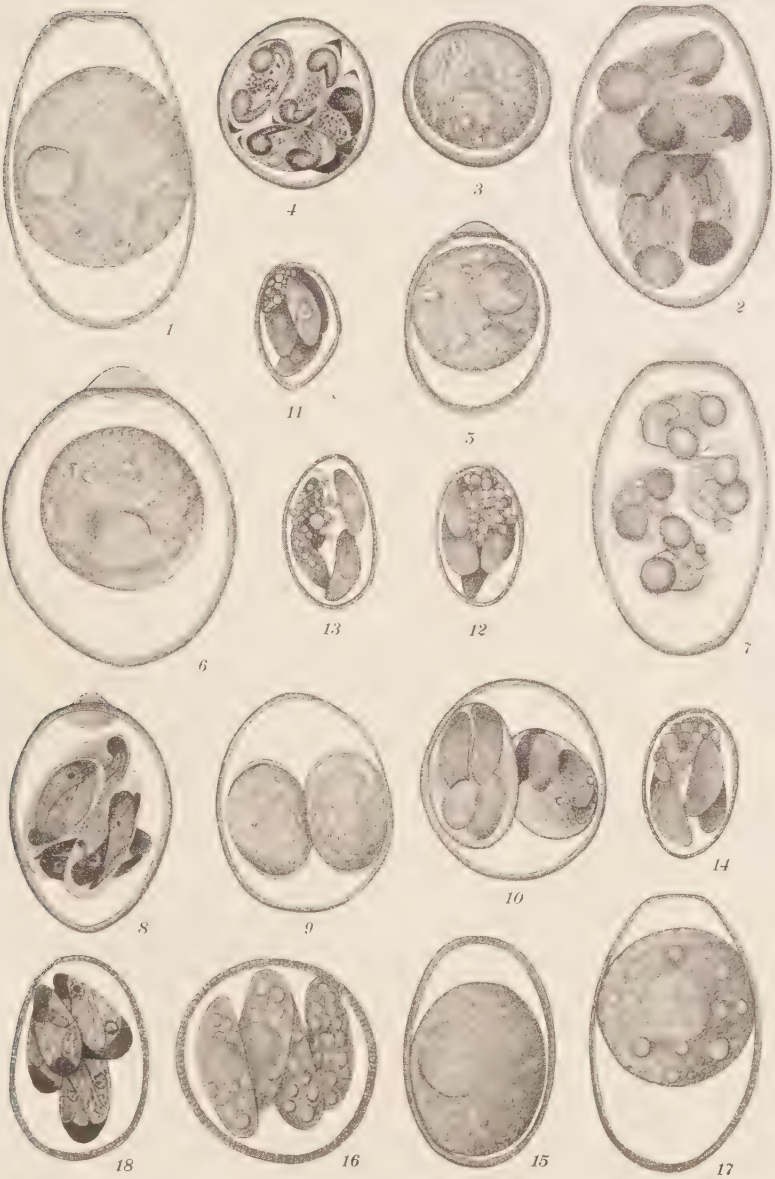


PLATE XIX

THE HEMOTOXINS OF INTESTINAL PARASITES. A CRITICAL SUMMARY WITH NOTES ON SOME CASES

JOSEPH LEIDY II

PHILADELPHIA

The mechanical and reflex disturbances produced by animal parasites in the intestinal canal and other organs of the body have long been recognized by pathologists and clinicians. The researches of economic parasitologists and the occasional reports by clinical observers indicate the presence of other factors deserving of further investigation. That certain parasitic worms secrete substances that effect the blood of their hosts deleteriously has been shown conclusively by the researches of Tallqvist, Schwartz, Schaumann and others. The broad tapeworm of man which is known to produce severe anaemia contains a hemolytic agent according to the experiments and researches of Schaumann and Tallqvist. Hookworms secrete a hemolysin and an anticoagulin according to Calmette and Breton, Loeb and Smith. The whipworm Trichuris apparently secretes a hemolysin according to the investigations of Whipple (1909) and Sarin (1913).

In 1865 Kuttner cites a case of blood destruction cured by expelling ascarides. According to Filatoff (1897) Karaven cured a case of pernicious anemia by expelling ascarides from the intestine. Francois (1906) in the course of his investigation of miners found many cases of severe anemia in which hookworms were not present, but which showed numerous ascaris eggs in the feces, and Schwartz has shown that the anemia in hogs and horses is frequently associated with *Ascaris lunbricoides* infection. Two views as to the cause of parsitic anemia have been held by different authorities. One that the anemia results from depriving the host of blood by sucking the other through the secretion of a hemolysin, a hemotoxin hemolytic in character. Owing to the fact that the abstraction of blood by parasites appears to be inadequate as an explanation of the causes of anemia in parasitic diseases, and in tapeworm infections due entirely to the presence of parasites, the direct abstraction theory as applied to hookworm anemia is inapplicable. From the researches of Schwartz and others the view that hemolysins from parasites are of etiological significance in parasitic diseases appears to be entirely justified.

Huber (1870) expressed the opinion that many symptoms put down to round worms are caused by a peculiar irritating matter which they

---

Read before the Section on Medicine, College of Physicians, Philadelphia. Feb. 26, 1923.

contain. Huber draws attention to the observation of Mirams that the examination of *Ascaris megalocephala* had twice caused him most unpleasant symptoms such as sneezing, swelling at the puncta lachrymalis, hypersecretion of tears with violent itching and swelling of the fingers. Huber states after a personal examination of twelve examples of *Ascaris lumbricoides* he suffered from troublesome itching of the hand and neck; over the latter the skin was raised here and there in lumps, smaller lumps appearing on the forehead. His right ear swelled up and for an hour a plentiful secretion came from the meatus associated with a most unpleasant sensation of pulsation in the right side of the head radiating from the ear. His conjunctiva became inflamed, accompanied with severe itching, the inflammation leading to chemosis in the right eye and finally itching of the hands. After the lapse of an hour these symptoms gradually subsided. Huber considers that these symptoms were caused by the peculiar substance which gives off the strong smell peculiar to round worms, and which as Leuckart supposes is contained in the vacuoles between the muscular fibers. In 1898 Schaumann and Tallqvist reported the discovery of a blood toxin in the broad tapeworm of man; four years later, 1902, Schimmelpfennig announced that the symptoms produced by the horse ascaris were due to a hemotoxin. He observed in the presence of the coelomic fluid of *Ascaris equorum,* red blood cells from the horse became cremated and were ultimately destroyed through a process hemolytic in character. It should be stated, however, in this research of Schimmelpfennig no mention of the percentage of alkalinity of the coelomic fluid is given.

Anemia has been frequently associated with the ascarides in both man and animals and the literature reveals the fact that this anemia has been mistaken for hookworm and pernicious anemia. The researches of Faust, Beumer, Dascotte, cited by Weinburg, Calamida, Kolmer, and more recently Ransom and others indicates the importance of a closer study by clinicians of these problems which have been confined more particularly to medical and economic zoologists. It has been shown that *Diphyllobothrium latum,* the broad tapeworm of man, is capable of causing severe anemia clinically indistinguishable in the opinion of various observers from pernicious anemia and according to these investigators differing from the former in one respect only, namely, by the disappearance of the symptoms and recovery of the patient after expulsion of the parasites. On the other hand there are numerous cases on record in which the presence of *Diphyllobothrium latum* in man was not accompanied by anemia. What then is the explanation of the immunity noticeable in one group of cases not present in the others? Schwartz has shown that the serological reaction of the hosts harboring parasites afford proof that parasitic worms liberate products against which the hosts develop defense or immunity reactions. Kolmer, Trist

and Heist according to the results of complement-fixation tests with the sera of infested dogs had reason to believe that production of antibodies may occur after infestation of the intestines with the common parasites.

Worms belonging to the genus Ascaris contain a hemolysin which is closely bound to the muscle tissues of the worm and is but slightly soluble in water (Schwartz). Other observers are of the opinion the toxin is given off from other structures and organs. Can it be in the former case one finds an explanation of the immunity of the host in those cases where there is an absence of profound constitutional disturbance? The ascarides appear also to secrete a feeble anticoagulin. In a series of experiments in vitro Schwartz found that extracts of the intestines of parasites were strongly hemolytic, whereas extracts from the body wall showed no hemolytic effect. Extracts of the reproductive organs were but moderately hemolytic. In a second series of experiments extracts of the intestines were strongly hemolytic whereas extracts of the reproductive organs and body wall showed weak hemolytic powers.

The present note has to do with the results of a clinical study of three cases of parasitic infection which have come under the author's observation and points strongly to the hemotoxin of the ascarides as an etiological factor deserving of attention.

Case 1.—Female, aged 12, applied for treatment April, 1921.

Four years previously suffered from corneal ulcers and malnutrition which illness covered a period of from five to six months; the corneal condition was markedly resistant to treatment so much so that the case was looked upon as one of incipient tuberculosis. The mother reports after six months' illness the child suddenly began to improve without apparent cause and in a very short time had recovered her former good health. The patient was referred to me as one of possible incipient tuberculosis. The corneal condition which had recurred was under the care of Dr. E. S. Saylor. The child was poorly nourished, there was loss of weight and color, from an active cheerful disposition, the patient was depressed, indisposition to play or take interest in her toys or books. Examination of the heart, lungs and genito-urinary tract proved negative. Blood examination: Hemoglobin, 70 per cent.; eosinophils, 14 per cent. Gastro-intestinal tract: Mother reports four years previously the child had passed a large number of dead worms, very offensive. She had administered a "worm medicine" purchased as a proprietary medicine when she detected the parasites in the stools but had not reported the fact to the attending physician. With this history taken in connection with the high eosinophil count (14 per cent.) santonin was administered which was followed by the expulsion of a coil of twelve worms to be followed in six hours by the expulsion of four worms all dead and presenting the appearance of being partially digested and decomposed.

The patient made a prompt and uninterrupted recovery. She is now in full vigor of health, blood count normal, no ova in stools and increased (21) twenty-one pounds in weight. The rapid recovery from both of these attacks following the expulsion of disintegrated or partially digested worms is significant.

Case 2.—L. R., male, aged 9.

With chronic eczema of eight months' duration, occasional epistaxis, and malnutrition. The patient had run the gauntlet of local and constitutional treat-

ment, physical examination of heart, lungs and genito-urinary tract negative; urine showed increase in indican, no increase in sulpho-ethers; microscopic examination of feces showed the presence of large numbers of ova of ascarides. Treatment: santonin was administered followed by the expulsion of twenty round worms, in one coil; there were three dead, disintegrated and partially digested. The day following the expulsion of the parasites the eosinophil count was 16 per cent.; hemoglobin, 70 per cent. Three months later the patient was free from all evidence of eczema; eosinophil count, 3 per cent. The patient is now well ten months after infection was discovered.

D. T. R. Brown in Johns Hopkins Hospital Reports cites a case of chronic eczema with an eosinophil count of 22 per cent. but no reference is made to the condition of the gastro-intestinal tract or results of treatment.

Case 3.—I saw in consultation with Dr. R. E. Brown of New York, March 3, 1911, male, aged 5. The subject of frequent attacks of angioneurotic edema, the symptoms were alarming to the family and attendants, the case presented a classical picture of the condition. Blood examination two weeks previous to consultation: esosinopils, 10 per cent.; hemoglobin, 70 per cent.

The condition was viewed as one of intestinal toxemia; there was no increase either of indican or sulpho-ether excretion. Microscopic examination of feces showed large numbers of ova of ascarides. The administration of santonin was followed by the expulsion of twenty-six round worms, many dead disintegrated or partially digested. There has been no recurrence of the attacks in twelve years. The boy is now in perfect health.

Intestinal toxemia is not unfrequently an etiological factor in cases of angioneurotic edema, but in all those cases which have come under observation of the writer the indican content of the urine ran high with an appreciable increase in the elimination of the sulphoethers and in no case was there an increase in the eosinophil count. The fact that in the cases reported three or more of the parasites were dead and showed evidence of having undergone decomposition, disintegration and partial digestion by the host is significant. To what extent hemotoxic substances are liberated when the parasites sicken or undergo degeneration deserves more extended investigation. In the case of the broad tapeworm, *Diphyllobothrium latum,* it appears highly probable the observation as pointed out by Schwartz that certain individuals may lack antilytic constituents in the blood and are thus susceptible to the toxin which other individuals are capable of neutralizing, may prove correct. On the other hand that parasites may die and undergo disintegration before elimination by the host is made clear from the cases cited.

Leidy (1849) demonstrated that parasitic worms while living within the host may harbor vast quantities of cryptogamic vegetation with a great variety of species of entophyta growing upon the surface of the living parasite. Weinburg described what appears to be a disease in worms belonging to the genus Ascaris, which is characterized by the presence of certain pigmented spots visible through the cuticula. This condition has also been observed by Schwartz. It would appear from these observations that both causes acting together or independent of the other may prove to be the prime etiological factor in the production

of a group of symptoms which were formerly attributed to mechanical or reflex phenomena. The presence of a high eosinophil count in the peripheral blood is generally considered presumptive evidence of parasitic infection by helminthologists. The literature has become extensive and with a long list of observers leaning strongly to the presence of hemotoxins as etiological factors in a group of symptoms which have been hitherto clustered under the shield of mechanical or reflex phenomena a new field is open for further investigation which bids fair to place what may be termed the parasitic syndrome as applied to intestinal parasites upon a workable scientific basis.

In view of the results obtained from the surveys made in our army camps for hookworm and allied parasites all cases especially among young children and adolescents presenting the picture of malnutrition, anemia with or without increase in the eosinophil count should be viewed with suspicion as being the subject of parasitic infection and a microscopic examination of the feces be made to determine the presence or absence of parasitic ova; indeed the time has arrived when the examination of the feces should be as much a matter of routine as the examination of the urine. In any health survey of school children the microscopic examination of the feces should become a part of the physical examination as was pursued in army camps during the World War.

Bibliography

Brown, T. R. 1898.—Studies on Trichinosis, with Especial Reference to the increase of the Eosinophilic Cells in the Blood and Muscle, the Origin of these Cells and their Diagnostic Importance. Jour. Exp. Med., 3: 315-348.

Calamida, Dante. 1901.—Weitere Untersuchungen über das Gift der Tänien. Centbl. Bakt., Abt. 1, 30: 374-375.

Calmette, A., and Breton, M. 1905.—L'ankylostoma. L'ankylostomiase. Paris. 246 pp.

Filatoff, N. F. 1897.—Vers intestinaux. *In* Traité des maladies de l'enfance de J. Graucher, J. Comby. Paris. Pp. 670-698.

Francois, E. 1906.—Anemie des mineurs. Paris. 122 pp.

Garin, Charles. 1908.—Trichocèphale gorgê de Sang. Lyon Med., 11: 383.

1913.—Recherches physiologiques. Ann. Univ. Lyon, n.s. I, sci. med. fasc. 34.

Guiart, Jules. 1914.—Biologie et role pathogene des parasites animaux. In Bouchard and Roger, Nouveaux Traité de Pathologie Générale, 2: 83-973.

Huber, J. 1870.—Parasites. Ziemssen Encylopedia, 7: 74.

Kolmer, J. A., Trist, M. E., and Heist, G. D. 1916.—Complement Fixation in Intestinal Parasitism. Jour. Infect. Dis., 18: 88-105.

Kuttner. 1865.—Mesenterial-neuralgie mit Fieber. Berl. klin. Wchnschr. 2: 308-309.

Loeb, Leo, and Smith, A. J. 1904.—The Presence of a Substance Inhibiting the Coagulation of the Blood in Anchylostoma. Proc. Path. Soc. Phila., n. s., 7: 173-177.

Leidy, Joseph. 1850.—Germs Living Within the Intestinal Canal of the Lower Animals. Proc. Acad. Nat. Sci. Phila., Oct., 1849; Jan., 1850.

1853.—Flora and Fauna Within Living Animals. Smithsonian Contrib. Knowl., 5, Art. 2, 67 pp.

Ransom, B. H. 1922.—Observations on the Toxic Effects of Ascaris Fluids. Jour. Parasit., 9: 42-43.

Schaumann, Ossian. 1898.—Ueber die Blutkörperchen auflösenden Eigenschaften des breiten Bandwurms. Deut. med. Wchnschr., 24: 312-313.

Schimmelpfennig, Gustav. 1902.—Ueber *Ascaris megalocephala.* Beitr. Biol. u. physiol. Chemie derselben, 46-49. Inaug. Diss., Berlin.

Schwartz, Benjamin. 1921.—Hemotoxins from Parasitic Worms. Jour. Agric. Res., 32: 8.

Tallqvist, T. W. 1907.—Zur Pathogenese der perniciösen Anämie mit besonderer Berücksichtigung der Bothriozephalus-anämie. Ztschr. klin. Med., Berl., 61: 427-532.

Weinburg, M. 1907.—Sur une hemotoxine d'origine vermineuse. Compt, rend. soc. biol. Paris, 63: 13-15.

1911.—Substances toxiques de *l'Ascaris megalocephela.* Compt. rend. soc. biol., Paris, 70: 337-339.

1912.—Une maladie de *l'Ascaris megalocephala.* Compt. rend. soc. biol., Paris, 73: 260-262.

A NOTE ON DR. GUNN'S DIFFICULTIES WITH COUNCILMANIA

Charles A. Kofoid and Olive Swezy

In a short article (1922) Dr. Herbert Gunn, at that time Assistant Visiting Physician at the San Francisco Hospital on the staff of Stanford University Medical School, gave his grounds, for concluding that our (1921) *Councilmania lafleuri* is only *Endamoeba coli* Loesch as Wenyon (1922) had previously concluded.

In the year referred to by Dr. Gunn we made for him among many other stool examinations requested, 29 of stools reported by him to be from 23 different persons in which we found either *E. coli, C. lafleuri,* or both. On certain stools he requested a report on *E. coli,* sometimes by telephone. We have no record of the serial number of those stools on which the report on *E. coli* was requested and made, and are unable therefore to distinguish them from stools in which we found *E. coli* but did not report it. In our ordinary routine in recent years no reports are made to physicians on *E. coli* since it is not regarded as pathogenic. Our records show that in the 23 "cases" based on the names submitted with the sample, we recorded at the time of the examination 10 cases of *C. lafleuri* (Gunn reports but 8), 8 of *E. coli,* and 3 of mixed infections.

The amount of time which can be given in ordinary laboratory routine to searching out infections of *E. coli* is limited by the pressure of the day's work which must be kept up to date. It is not our routine to push the search for *E. coli,* nor to report any findings when the cysts are such as to render diagnosis difficult, but to ask for an additional stool if the indications suggest *E. dysenteriae. E. coli* in our experience is not often abundant in the stool and of all the common species it is most wont to be overlooked when slides are not widely searched. In 1600 stools treated by the Carles-Barthelemy centrifuge method at the Army Laboratory in 1918 we detected a trifle over 10 per cent. increase in *E. coli* above findings by the smear method.

Furthermore, many of the samples submitted by Gunn were manifestly old when they reached us and were sometimes moldy. In stale stools we find that *Councilmania* cysts, perhaps even more than those of other amoebae, tend to disappear. Stale stools also contain, as a rule, a larger proportion of cysts with abnormal nuclear conditions which render diagnosis more difficult. We withhold reports as a routine on all doubtful cysts. Our attempt to secure an additional stool of a case submitted by Gunn was unsuccessful. Fortunately we still have stained slides from 22 of Gunn's cases. We have gone over the whole or a

sufficient area of each of these slides with care, with the result that we find both *E. coli* and *C. lafleuri* are present in 18 of the 22 cases. In 3 of the 4 remaining cases we had only a single stool. *C. lafleuri* alone was present with certainty in three of these and *E. coli* in the other one.

From these facts it appears that 18, if not all, of the "cases" were ones of mixed infections. It is our judgment that many of Gunn's difficulties arise from the fact that he was dealing with and comparing mixed infections. Had we known of his purpose we should of course have sent to him our full findings in every case, and would have pushed our examination farther than is customary in the matter of *E. coli* in our usual laboratory routine. Gunn's criticisms are based in large part on his inability to distinguish the amoebae in these mixed infections. His treatment befogs the matter in that in his discussion he primarily compares cases rather than amoebae or cysts. It is furthermore difficult to treat the paper critically because of general statements and lack of figures.

The following is a brief summary of our findings on this subject since the publication of our first account (Kofoid and Swezy, 1921) in the main included in recent papers (Swezy, 1922; Kessel, 1923a, b; Kofoid, Swezy, and Kessel, 1923). We have since our discovery of Councilmania made 20,264 stool examinations on 4763 persons. In them we found 373 cases of *C. lafleuri* or 7.83 per cent., and 541 of *E. coli* or 11.35 per cent. There were 192 cases of coincident infection of the two species detected in our normal routine.

We have examined one case, a clinically cured case of infection by *E. dysenteriae,* 113 times after treatment with 700 stained slides at our disposal without a single cyst of *E. dysenteriae* being found. This case had after treatment and clinical cure of *E. dysenteriae,* an infection of *C. lafleuri* with typical cysts and motile amoebae with *clear* pseudopodia. Some of these amoebae contained red blood corpuscles. Gunn would have his readers believe that these amoebae were *E. dysenteriae* though no cysts of it occurred in the case, and that we mistook vacuoles for corpuscles. We also had another case under examination for over a year with 78 examinations in none of which was *E. dysenteriae* ever seen. This case had the cysts and the motile amoebae with clear pseudopodia of *C. lafleuri.* Many other case records of similar import but less extensive are available in our data.

Furthermore, Kessel (1923a) has established and tested out a method of obtaining amoeba-free rats and has infected them experimentally with *C. lafleuri,* with *E. coli,* and with mixed infections of the two from man. These infections have been tested in the rats by fecal examination and at autopsy with confirmatory results. In these transfers and in subsequent ones from rat to rat the pseudopodia, nuclei, and number of chromosomes remain demonstrably constant. *C. lafleuri* has

eight chromosomes and *E. coli* has six. The pure infections run true to type and the mixed ones in the rat contain the motile amoebae and the cysts of both types.

Our observations on the pseudopodia of human intestinal amoebae on the electric warm stage with regulated temperature, and specifically on *E. coli* and *C. lafleuri,* do not accord with Gunn's dictum that in the examination of pseudopodia and motility one "is liable to great error," unless one be careless or inept. The pseudopodia may be studied (in characteristic appearance and behavior) in such fresh smears for several hours. Under these conditions we find that *E. coli* has granular pseudopodia continuously and, in comparison with *C. lafleuri* on the same slide or in similar conditions, is less active.

Gunn's failure to find red blood cells in *C. lafleuri* + *E. coli* is no proof that under suitable conditions *C. lafleuri* does not ingest these cells as we have stated. No competent cytologist would "readily and frequently" mistake vacuoles for red blood cells as he intimates we have done. The erythrocytes which we have found in *C. lafleuri* in fresh smears and in stained slides are typical in size and in color, in staining reactions, and in metabolic changes. His statement that "there appeared to be no difference in the thickness of the cyst walls in Councilmania and the check cases" is due to his mixed infections. One must measure these walls in specifically determined cysts to establish this contrast. This comparison is best made in fresh smears. His failure to find differences in contour is due to comparisons of mixed infections and his statement that "asymmetrical forms were readily produced by pressure on the cover slip" probably rests on the fact that pressure usually turns or rolls the cysts and thus reveals the ellipsoidal form of some cysts. In our experience spherical cysts do not lose their circular outline on pressure.

Gunn finds no differences in stainability between the cysts of Councilmania and *E. coli* "check" cases. This again rests on his comparisons of cases of mixed infections. In our experience in examining stained slides of fecal smears (over 20,000 in our collection) the cysts of Councilmania more frequently than all others resist the stain. The use of hot fixer and warm stain removes much of this difficulty. Studies (not yet published) on the resistance of cysts to reagents indicate a wide variation in resistance within the species, and also in the same stool and in the same case. This is probably due to the ages of the cysts and to the stages in the development of the wall at which the cysts are fixed or examined. It is our experience that non-budding cysts of *C. lafleuri* resist both the iodine-eosin stain and cold fixer and subsequent staining operations much more successfully than do the cysts of any other amoeba in the fecal smears. In some slides and some stools this is much more evident than in others.

The statement that there was "no difference in the appearance of the karyosome in the Councilmania and *E. coli* cases" again rests on mixed infections. In our slides of Gunn's cases there exist the cysts of the two types with the differences we have described between *C. lafleuri* and *E. coli*. *E. coli* has a small, solid, spherical, excentric karyosome. *C. lafleuri* has a large, usually central karyosome made up of dispersed granules. In some cysts in the prophase these are more massed, and move through an excentric position as they pass peripherally to a location on the nuclear membrane before division. In depauperate or degenerating cysts in stale stools the karyosome may be smaller than usual, but it is even then normallly central and exhibits traces of its granular organization on its periphery if properly decolorized. In addition to this in old cysts with 8 or 16 nuclei or budding cysts with 8 to 1 or 16 to 1 nuclei the peripheral chromatin is often very thin and the intermediate zone often remarkably clear. *E. coli* tends to have more peripheral chromatin and its nuclei stand out less clearly as light areas in the cytoplasm than those of *C. lafleuri* in many if not in most cysts. During prophases these conditions are modified.

Gunn's statement that there was "no difference in the chromatoidal bodies" in the two "check cases" again rests on mixed infections. The difference between these bodies in the cysts of the two amoebae is clearly traceable in mixed infections and is confirmed by our studies of supposedly pure infections in man and in the same when experimentally introduced into the rat. The chromatoidal bodies of *C. lafleuri* are more flake-like or chip-like and in late stages of the cyst tend to mass in the center in a single bundle. In *E. coli* they are more splinter-like, and tend to be more dispersed throughout the cytoplasm of the cyst. These are, however, variable and evanescent structures and present a very wide range of number, frequency, form and arrangement, but even under these conditions the distinctions will often appear in cysts of comparable stages and amounts of the chromatoidal substance. Gunn's conclusions that the budding we describe is an artefact seems to rest on the following arguments, (1) he had not previously seen budding; (2) he did not find it in his preparations of the stools which he sent to us; (3) he produced evacuation of cysts by pressure. No one of these facts is conclusive or valid evidence that budding does not normally occur in Councilmania.

It is, of course, possible to evacuate cysts in which the pore is already present and to crush cysts by pressure. These two results give different pictures (see Kofoid, Swezy, and Kessel, 1923). *Post hoc, ergo propter hoc* is his argument for all the budding cysts he reports. In our experience the facts are that budding cysts occur in warm fresh stools untouched by pressure, in the cecal contents of the rat at autopsy, in

fresh stool fixed without the least pressure and sectioned, in cysts on slides from Gunn's cases in which the cyst is not in contact with the slide or with the cover. In some cysts the bud is on the top. His sweeping universal statement that "this deeply staining ridge is clearly brought about in all the specimens where budding was produced by pressure and it resulted of course from the entrance of the stain into the creased wall of the ruptured cyst" is in our varied experience wholly without critical evidence or cytological foundation. As a matter of fact, the wall of the normal, of the budding, or of the crushed cysts does not take the stain. It is the cytoplasm which stains and the chromophile ridges and the buds are normal biological phenomena which occur normally and independently of pressure, in *C. lafleuri* but not in *E. coli*. They are cytoplasmic structures staining differentially and are never composed of ruptured or wrinkled wall. We fear Gunn has misinterpreted what he saw and has drawn conclusions unsupported by the evidence before him, and are confident that our observations are confirmable with due precautions as to method and to cytological analysis.

A clear understanding of the distinctions between *Endamoeba coli, Councilmania lafleuri,* and *Endamoeba dysenteriae* is a matter of great importance in the clinical diagnosis of amoebiasis. In the past it has been the custom of clinical microscopists to diagnose amoebae with clear pseudopodia and especially those containing red blood corpuscles as the motile stage of *E. dysenteriae*. It is obvious that in the light of our findings on *C lafleuri* that this basis of diagnosis does not in itself distinguish *E. dysenteriae* from *C. lafleuri*. Such diagnosis should be based upon the cysts. We therefore regard the conclusions of Wenyon and of Gunn not only as unsound scientifically but as liable to lead to false diagnoses.

Zoological Laboratory, University of California.

Literature Cited

Gunn, Herbert. 1922.—*Councilmania lafleuri* not a New Amoeba. Jour. Parasit., 9: 24-27.

Kessel, J. F. 1923a.—Methods of Obtaining Amoeba-Free Rats for Experimental Infection with Intestinal Amoebae. Univ. Calif. Publ. Zool., 20: 401-408.

1923b.—Experimental Infection of Rats and Mice with the Common Intestinal Amoebae of Man. Univ. Calif. Publ. Zool., 20: 409-430.

Kofoid, C. A., and Swezy, O. 1921.—On the Free, Encysted, and Budding Stages of *Councilmania lafleuri,* a parasitic amoeba of the human intestine. Univ. Calif. Publ. Zool., 20: 170-189.

Kofoid, C. A., Swezy, O., and Kessel, J. F. 1923.—On the Genus Councilmania, budding Intestinal Amoebae Parasitic in Man and Rodents. Univ. Calif. Publ. Zool., 20: 431-445.

Swezy, O. 1922.—Mitosis in the Encysted Stages of *Endamoeba coli* (Loesch). Univ. Calif. Publ. Zool., 20: 313-332.

Wenyon, C. M. 1922.—On Three Supposed New Entamoebae of Man. Trop. Dis. Bull., 19: 19-22.

NOTES ON TWO NEW MONOSTOMES WITH RUDIMENTARY VENTRAL SUCKERS

Kaoru Morishita
Zoological Institute, Faculty of Science,
Tokyo Imperial University

Haematotrephus phaneropsolus Stossich 1902, which according to Kossack is possibly synonymous with *Cyclocoelum brasilianum* Stossich and was taken from *Totanus sp.* in Yedo (Tokyo), is the only species of monostomatous trematodes so far described from Japan, although certain others are known as occurring in this country. To *Cyclocoelum mutabile* (Zeder) I have referred a specimen collected by Taguma, a veterinarian, from the body cavity of *Gallinula chloropus* in Tokyo. In the collection of the veterinary pathological department of the Tokyo Imperial University, there is a bottle labelled *Monostoma verrucosa* (= *Catatropis verrucosa*) from the chicken. Sugimoto (1919) listed two species of Typhlocoelum in his list of Zooparasites of the domesticated animals in Formosa, i. e., *Typhlocoelum flavum* (Mehlis) from the trachea and esophagus of the domestic duck and some other water birds, and *Typhlocoelum hepaticum* Sugimoto (1919) from a hepatic cystoma of the same host animal.

The present paper deals with two species of Cyclocoelum from the birds which have long been preserved in this Institute. According to my observations they are not only new to science but of especial interest in possessing a rudimentary ventral sucker, as may be seen from the following descriptions. I wish to take this opportunity to express my hearty thanks to Prof. S. Goto for his constant supervision and valuable suggestions during the progress of my work.

Cyclocoelum vagum n. sp. (Fig. 1-5)

Diagnosis.—Middle sized worms, 9-10 mm. long by 3-3.5 mm. in maximum width which lies in the posterior third of the body. Oral sucker weakly developed, ventral sucker very rudimentary. Pharynx large and spherical; esophagus short, coiled dorsoventrally. Genital pore at center of anterior intestinal arch. Cirrus pouch short, tadpole-like, lying for the most part behind the intestinal bifurcation. The three genital glands arranged in a straight line in the order testis-ovary-testis. Yolk glands extending in front as far as level of posterior margin of cirrus pouch, behind to level of posterior intestinal arch. Uterine coils not reaching inner edges of intestinal curura, or extending a little beyond. Eggs, 67 to 77μ long by 37 to 43μ broad; enclosed embryos without eye spots.

Habitat.—Ductus nasolacrymalis of Golden pheasant, *Chrysolophus picta* (L).

Nine specimens of this species, preserved in alcohol and collected by Yoneyama, March 12, 1899, were available for study. The locality

is not stated. The host birds occur naturally only in western China, but have been introduced and widely domesticated in Japan for more than three hundred years. I am, therefore, unable to assert whether the actual source is China or Japan.

The flukes are of medium size, flat but fleshy, measuring 9 to 10 mm. long by 3 to 3.5 mm. in maximum width which lies at the anterior part of the posterior third of the body, from which point the body narrows anteriorly gradually to the roundly pointed end and posteriorly to a broadly rounded end. The subterminal mouth is surrounded by a musculature so weakly developed as hardly to deserve the name of a sucker. The ventral sucker, situated a little behind the anterior intestinal arch and about 0.54 mm. from the oral opening, is very rudimentary but possesses definitely the structure of a sucker, though it may not be functional, and is about 0.05 mm. long and 0.1 mm. wide, as measured in serial sections of a specimen. It is difficult to make out its presence in total mounts. The mouth cavity is funnel-shaped, somewhat flattened from side to side, separated from the pharynx by a very short prepharynx. The well developed pharynx is spherical in shape, with the diameter of about 0.29 mm. The short esophagus is coiled dorsoventrally and almost embraced by the wall of the anterior intestinal arch, so that in total mounts one may easily be misled to believe in its absence. The voluminous, more or less irregular intestinal crura run almost parallel to the margin of the body and as in other species of this genus, are continued into each other posteriorly. The anteroposteriorly flattened excretory vesicle is situated behind the posterior intestinal arch and opens to the exterior through a very short duct, by a small dorsoterminal pore. The lateral branches run along the intestinal crura to the level of the pharynx. The lymph system, of which I can not find any previous account in this genus, is composed of four main canals, which pass two by two along each intestinal crus as far as the region of the pharynx, giving out on the way numerous small branches, which anastomose with each other to form a mesh-work; in addition there is a comparatively large vesicle on the dorsal and posterior side of the excretory vesicle, sending out numerous diverticula around the latter.

The genital glands are situated in the posterior fourth of the body. The posterior testis, in contact with the inner margin of the posterior intestinal arch and situated somewhat to the right of the median line, is flattened anteroposteriorly and measures 0.58 mm. long by 0.87 mm. wide. The posterior vas efferens takes rise from the right anterior end of this testis. The anterior testis, contiguous to the left intestinal crus and separated from the posterior always by three uterine loops and the ovary, is flattened laterally, measuring 0.84 mm. long by 0.69 mm. wide. The anterior vas efferens emerges from its left anterior end.

The cirrus pouch is short and tadpole-like in its form. The large seminal vesicle, measuring about 0.19 mm. long by 0.29 mm. wide, occupies the greater part of the pouch and is continued anteriorly into a retort-like prostate region, which is again followed by a narrow, relatively long, much coiled duct inside the pouch. This terminal duct opens into the comparatively long genital atrium, the exterior aperture of which lies in the median line at the level of the anterior intestinal arch. The part of the cirrus pouch containing the seminal vesicle is situated posterior to the intestinal bifurcation.

On the left side of and anterior to the posterior testis is the ovarian complex. The ovary is situated in the straight line passing through the centers of both testes and measures 0.27 mm. long by 0.33 mm. wide. The shell gland, almost as large as the ovary itself, lies on the left side of the median line, posteriad and dorsad to the latter organ. The oviduct is very short, and immediately after its entrance into the shell gland it is enlarged to form a sac-like diverticulum, which may be the same organ as that Harrah (1922) has designated as the receptaculum seminis. For various reasons, however, I am inclined to regard this organ as the ootype. After this enlargement the oviduct passes through the shell gland as a narrow tube without showing any part corresponding to what Harrah has called the ootype. In the diverticulum there are seen many yolk cells. These facts lead me to the opinion that the diverticulum is not a receptaculum seminis but the ootype. It occupies the left anterior part of the shell gland, and lies partly outside the latter.

The greater part of the vitellaria lies between the lateral margin of the body and the intestinal crura, rarely overlapping the outer part of the latter. In the posterior part they shift over to the ventral side of the intestine. They extend anteriad almost to the level of the posterior margin of the cirrus pouch and posteriad to the level of the posterior intestinal arch. The paired vitelline ducts lie in the region of the posterior testis, the right one proceeding from its origin slightly anteriad but mainly transversely to the anterior end of the posterior testis and then slightly curving posteriad, continuing its course to the point of union with its fellow directly behind the shell gland. The left one is only about one-third as long as the other. The common duct formed by their union penetrates into the shell gland after a short course and finally opens into the ootype from the right posterior side. From this point of union arises another narrow duct which passes through the gland and on leaving it at its left posterior corner, is enlarged into the receptaculum seminis uterinum. Laurer's canal is absent. The crowded uterine loops entirely fill up the posterior two-thirds of the space between the intestinal crura, but are somewhat loose in the anterior one-

third. The loops are almost exactly transverse and do not extend beyond the inner margin of the crura, or in some instances, only a little beyond. The metraterm is strongly muscular and provided with some gland cells around it. The eggs are long and elliptical, 67 to 77μ long by 37 to 43μ wide. No eye spots have been seen.

Cyclocoelum distomatum n. sp. (Figs. 6-9)

Diagnosis.—Flat, elongated trematodes, 5.5 to 8 mm. long by 3 to 3.5 mm. in maximum width which lies in middle of posterior half of the body. Oral sucker well developed, ventral sucker rudimentary. Pharynx elognated, muscular, almost as large as oral sucker. Esophagus S-shaped. Genital pore at center of anterior intestinal arch. Cirrus pouch nearly spindle-shaped, lying for the most part behind intestinal bifurcation. The three genital glands arranged in a straight line in the order testis-ovary-testis. Yolk glands extending anteriorly to level of intestinal bifurcation, posteriorly to level of posterior margin of intestinal arch. Uterine loops with their outer ends directed more or less posteriad, and sometimes extending beyond the inner edges of the intestinal crura. Eggs, 50 to 60μ long by 30 to 40μ wide; enclosed embryos without eye spots.

Host.—*Phasianus, scintillans* Gould.

Numerous specimens of this species were collected from *Phasianus scintillans* in 1884, but no other records are available. The host bird may be, however, from Japan, as it is very commonly found in this country. As to the infested organ nothing is known, but the writer supposes from the well developed condition of the oral sucker and the presence of a rudimentary ventral sucker, that it was some organ opening to the exterior, e. g., digestive tract, trachea, or nasal cavity.

The worms are flat and elongated, 5.5 to 8 mm. long and 1.8 to 3 mm. in maximum width which lies in the middle of the posterior half of the body, from which point forward the sides are almost parallel up to the anterior one-third, which tapers gradually to a rounded point. The posterior end is obtusely rounded. The skin is thicker ventrally than dorsally. The oral sucker shows a well developed musculature and is nearly as large as the pharynx. The ventral sucker is more conspicuous than in the preceding species, though also rudimentary and possibly functionless, and lies about 0.5 mm. behind the oral sucker. It is occasionally seen even in total preparations as a round depression, measuring 53 to 60μ long by 60 to 63μ broad. The oral opening is subterminal and its funnel-shaped cavity leads directly into the pharynx, which is elongated and muscular with a length of 0.18 mm. and a breadth of 0.107 to 0.13 mm. The coiled esophagus is about 0.3 mm. long. The intestine is narrow and more or less irregular. The dorsoventrally flattened excretory vesicle, which lies between the intestinal arch and the posterior margin of the body, opens to the exterior by a terminal pore, provided with a sphincter.

The genital glands are situated in the posterior one-fourth of the body. The testes are generally spherical or ellipitical, but sometimes lobed. The posterior testis situated at the right corner of the posterior intestinal arch measures about 0.39 mm. long by 0.29 mm. broad. The anterior testis situated close to the left intestinal crus and separated from the posterior by two or three uterine loops and the ovary, lies on the left side of and posterior to the latter. The ootype occupies the ventromedial part of the shell gland, partly outside it.

The yolk glands extend anteriorly to the level of the anterior intestinal arch or sometimes further forward, and posteriorly to the level of the posterior intestinal arch. The vitellaria are separated from the lateral body wall by some space and limited on the inside by the outer wall of the intestine, shifting over occasionally only in the hindmost part on to the ventral side of the intestine. The fine paired yolk ducts lie in the region of the posterior testis, the right one passing along its anterior margin, then curving posteriad and finally uniting with the left one at the posteroventral margin of the shell gland. The left duct runs in a nearly transverse direction. The mode of connection of the various ducts of the ovarian complex is similar to that of the preceding species. The uterine loops almost fill up the space between the intestinal crura and sometimes overlap slightly their inner walls, and many of the loops have their outer ends directed somewhat posteriad. At the level of the cirrus pouch the metraterm runs almost dorsoventrally and opens into the genital atrium. The eggs are elliptical, 50 to 60μ long by 30 to 40μ wide; the enclosed embryos are without eye spots.

DISCUSSION

The two species described above differ from all the known species of the genus in the position of the genital pore and the arrangement of the genital glands. In these respects they do not even conform to the characters of the family Cyclocoelidae, if the diagnosis adopted by Kossack (1911) and amended by Harrah (1922) is used. In other respects, however, they show a close affinity with Cyclocoelum and I am of the opinion that the limits of the genus and the family should be extended to include these new species.

Concerning the arrangement of the genital glands there are three types in the genus as thus extended.

A. *Mutabile* type. (Text-fig. *a*) The most common type, in which the ovary is situated between the testes, and the three glands forming the points of a triangle: *C. mutabile* (Zeder), *C. microstomum* (Creplin), *C. obscurum* (Leidy), *C. problematicum* Stossich, *C. ovopunctatum* Stossich, *C. vicarium* (Arnsdorff) = *C. fasciatum* Stossich, *C. orientale* Skrjabin, *C. elongatum* Harrah, *C. obliquum* Harrah,

C. leidyi Harrah, *C. pseudomicrostomum* Harrah, *C. halli* Harrah, *C. cuneatum* Harrah, *C. macrorchis* Harrah.

B. *Vagum* type. (Text-fig. *c*) The ovary is situated between the testes, the three glands lying in a straight line. This type is considered to be a derivative of the *mutabile* type: *C. vagum* Morishita, *C. distomatum* Morishita.

C. *Taxorchis* type. Both testes are situated posterior to the ovary, the three glands forming the points of a triangle. There are two modifications. (1) The testes lying transversely on the same level (Text-fig. *c*): *C. taxorchis* Johnston, *C. wilsoni* Harrah, *C. triangularum* Harrah. (2) The testes lying obliquely (Text-fig. *d*): *C. tringae* (Brandes), *C. brasilianum* Stossich.

The foregoing types are based only on the mode of arrangement of the genital glands, and no other common differences can be pointed out, that will justify the separation of the genus into subgenera. Harrah has pointed out certain characters as being common to species forming my *taxorchis* type, but these are not found in all of them The *mutabile* type presents very miscellaneous characters. Only in the *vagum* type is there a common point of note in the position of the genital pore; but this again appears to me not sufficient to justify the establishment of a separate genus, because all the other characters are those of Cyclocoelum.

The view that the monostomes are of polyphyletic origin, has been suggested by several investigators. Cohn (1904) found in *Typhlocoelum flavum* a well developed but small ventral sucker, and proposed to transfer this species to Fasciolidae on the ground of this single character. Fuhrmann (1904) described a species with a well developed ventral sucker, *Bothriogaster variolaris,* stating that this on one hand is closely related to *Monostomum oculobium* Cohn (= *Spaniometra oculobia*) and on the other hand to Progonus Looss among the Fasciolidae. He would rather refer Brothriogaster to the Syncoelinae under the Fasciolidae, although the genus is at present placed in the Cyclocoelidae. These citations suggest the true relation between the monostomes and the distomes. The genus Cyclocoelum has been considered to be a most typical monostome group. My discovery of the ventral sucker in this genus has, however, proved that no very great weight should be placed on the mere absence or presence of the ventral sucker and that there is no distinct natural limit between the monostomes and the distomes.

Literature Cited

Brandes, G. 1892.—Revision der Monostomiden. Centrlbl. Bakt. Par., 13: 59-68.
Cohn, L. 1904.—Helminthologische Mitteilungen. II. Arch. Naturg., 1: 229-251.
Fuhrmann, O. 1904.—Neue Trematoden. Centrlbl. Bakt. Par., Abt. 1, Orig. 37: 58-64.
Harrah, E. C. 1921.—Two New Monostomes from Asia. Jour. Par., 7: 162-165.
1922.—North American Monostomes, Primarily from Fresh Water Hosts. Illinois Biol. Monogr., 7: 225-324.
Johnston, S. J. 1916.—On the Trematodes of Australian Birds. Jour. Proc. Roy. Soc. N. S. W., 50: 187-261.
Kossack, W. 1911.—Ueber Monostomiden. Zool. Jahrb., Syst., 31: 493-590.
Skrjabin, K. 1913.—Vogeltrematoden aus Russisch Turkestan. Zool. Jahrb., Syst., 35: 351-388.

Explanation of Plate XX

Figs. 1-5.—*Cyclocoelum vagum* n. sp.

Fig. 1.—Ventral view of entire worm. × 14.

Fig. 2.—Sagittal section through anterior body, showing pharynx, esophagus, intestinal arch, and positions of genital pore and rudimentary ventral sucker. × 57.

Fig. 3.—Transverse section through ventral sucker. × 240.

Fig. 4.—Cirrus pouch and metraterm. × 55.

Fig. 5.—Semi-diagrammatic figure of ovarian complex, drawn from paper reconstruction.

Fig. 6-9.—*Cyclocoelum distomatum* n. sp.

Fig. 6.—Ventral view of entire worm. × 14.

Fig. 7.—Ventral view of anterior end, showing position of ventral sucker. × 33.

Fig. 8.—Transverse section through oral sucker. × 160.

Fig. 9.—Longitudinal section through ventral sucker. × 250.

Abbreviations

es, esophagus
gp, genital pore
ld, lymph duct
od, oviduct
ot, ootype
ov, ovary
mc, mouth cavity
ph, pharynx
rsu, receptaculum seminis uterinum
sg, shell gland
sv, seminal vesicle
vd, vitelline duct
vs, ventral sucker

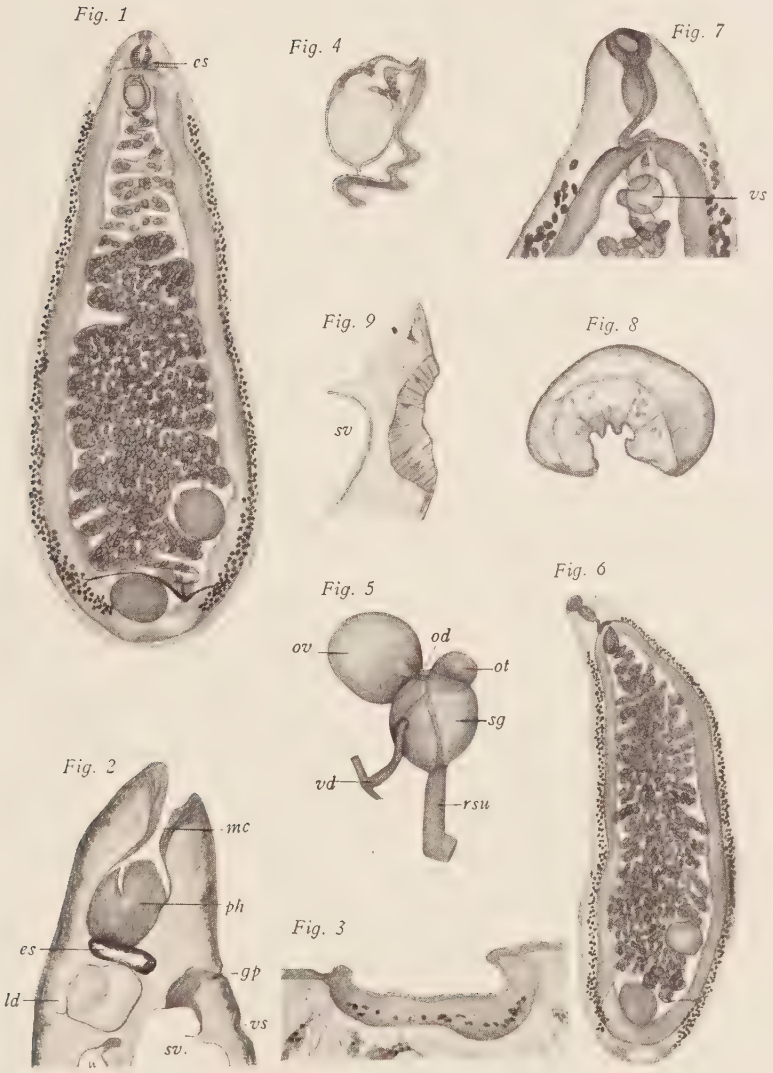


Fig. 1
cs
Fig. 4
Fig. 7
vs
Fig. 9
sv
Fig. 8
Fig. 5
ov
od
ot
sg
vd
rsu
Fig. 6
Fig. 2
mc
ph
es
gp
ld
vs
sv.
Fig. 3

PLATE XX

ISAO IJIMA

THE FATHER OF PARASITOLOGY IN JAPAN

(With Potrait Plate)

SADAO YOSHIDA
Pathological Department, Medical College, Osaka, Japan

It is a source of deep regret for us that the death of Professor Doctor Isao Ijima has taken from Japan one of the most famous founders as well as one of the most prominent leaders of zoology in this country. He was not only a most distinguished investigator himself, but also a great leader in zoology for all junior zoologists in our country. This is easily shown by his splendid works on various subjects and by the valuable papers of many of his fellow workers which he inspired and directed.

He was born as a son of Samurai at Hamamatsu, Shizuoka prefecture, on June 17, 1861. Under his father's influence he became much interested in foreign languages and science, and this led him to choose a course in pure science as his life work. At the age of 15 years, he entered Kaisei-Gakko (a preparatory course in the college) and in 1878, at 18 years of age, proceeded to the Science College of Tokyo Imperial University. After three years he graduated (1881) and immediately was appointed assistant in the College. In the following year he was ordered to go to Germany to study zoology at the University of Leipzig where he spent three years working under the direction of Professor Doctor R. Leuckart whose great influence led him to follow the study of parasitology after he returned home. He was granted the degree of Ph.D. by the University at the age of 24 years (1884). On returning to his native land in 1885 he was appointed the Professor of Zoology in Tokyo Imperial University where he remained up to the date of his death, a period of 36 years.

His numerous works on various subjects are generally classified under six topics: (1) Hirudinea, (2) birds, (3) Turbellaria, (4) parasites, (5) sponges and (6) text books. His interest in studying leeches originated in the great inspiration and kind guidance given by his teacher, Professor C. O. Whitman, an authority on that subject who was at that time in charge of zoology at Tokyo. He made an excellent collection of leeches, especially of the land leeches, during some years after his return from abroad. He was succeeded in this study by Professor A. Oka who afterwards published extensive works on Japanese leeches.

At about the same time he devoted himself to work for the advancement of ornithology in Japan and was much interested in collecting as well as in studying birds. During many years he worked on the splendid collection of birds which is one of the greatest objects of pride in our College Museum. Recently he was elected the president of the newly organized Ornithological Society of Japan.

In Germany he began the study of Turbellaria. The results of his elaborate investigations in this field were published in many articles, one of which constituted his thesis for the doctorate at the University of Leipzig. He continued the same study for some years after his return to Japan, but in hope of its completion the work was transferred to his students M. Eri and T. Kaburagi who have already published some articles in this field.

His last work was studies on sponges. Since 1893 he had been devoting himself to this subject and had published many valuable papers, especially the article *Studies on Hexactinellida, Contribution 1,* published in 1901, which was received with great admiration by all authorities in this branch of zoology. This world wide reputation led Dr. Weber, the director of the Siboga Expedition, to turn over the entire collection of sponges to Professor Ijima for study. He made great efforts to complete this work on Hexactinellida which demanded intensive study for many years. Up to the very moment of his death, he continued to prosecute this work and fortunately had nearly completed it. Mrs. Ijima recently told me that his death was surely hastened by over-work. Every day he studied till late into the night, in spite of her kind advice to take care of his health and not to exhaust his energy by over-work.

Undoubtedly his greatest contribution to zoology as well as to medicine in Japan was in his work on parasitology. Indeed he was the founder of parasitology in Japan. When he came back from Germany in 1885, nothing had been done in the investigation of Japanese parasites, although there were evidently many important questions to be investigated. He was the first lecturer on parasitology in our University, the first investigator as well as the first director of work on parasites in Japan, and the first author of books on parasitology in the Japanese language. For several years after 1885 he was engaged mainly in studying parasites and made numerous important discoveries in morphological and developmental lines as his papers show. It is a well known fact that he swallowed the larvae of the broad tape worm of man to prove the real source of this species in Japan. Not only did he himself cultivate assiduously the field of Japanese parasitology, but he led many younger men to continue the cultivation of this field of science. Among these Prof. S. Goto, Dr. M. Koidzumi, Dr. H. Kobayashi and others,

J. Jima

(1861—1921)

as successors to the late Professor, are the most prominent parasitologists in Japan and are engaged in carrying on numerous important studies on parasites.

His enthusiasm in work gave the deepest inspiration to those fellow-workers who came to study any subject under his direction. He was very kind to all with whom he came in contact, especially his fellow workers, and was always thinking and planning for their good or trying to assist his juniors to success in their work. It is difficult to give adequate expression to the feeling of sorrow at his death and to the loss which the Japanese Zoological Society has sustained. Though he is dead, his name will ever live in his works and in the hearts of us who were privileged to be acquainted with him.

Although deeply absorbed in zoological study as well as in guiding his students, he was not merely a scientist of keen observation, but he was also a man of charming personality, frank in character, and an interesting talker, characteristics which made him beloved by all who came in contact with him. He had many interests in life and was an enthusiastic lover of hunting and fishing. He was a famous sportsman, a good shot and his love of hunting and his interests in ornithology were closely related. He enjoyed his glass of wine and loved his pipe, which was always in his mouth.

He died on March 14, 1921, at the age of sixty-one years.

BOOK REVIEWS

LEHRBUCH DER MEDIZINISCHEN ENTOMOLOGIE. By E. Martini. Gustav Fischer, Jena, 1923. 462 pp., 244.

As the author well remarks in his preface while there have been many books of all sorts dealing with entomology there is no text dealing with the phase peculiarly related to medicine although this aspect of applied entomology has in most recent times come to be an extensive and important field of work. The four years which the author has spent on the preparation of the present book have resulted in the production of a masterful treatise. Its contents are arranged with reference to the medical significance of the material. One finds at first three chapters: on general structure of the group, on *Anopheles bifurcatus* as an example of its detailed morphology, and on a taxonomic survey of the arthropods. The author then considers in separate sections the arthropods as poisonous animals, as parasites, and as disease carriers, closing with an unusual and well developed discussion of means for the eradication of these animals. Naturally much the largest part of the book is devoted to the questions of parasites and of disease carriers, each of which topics is fully and intensively treated. It is not too much to say that the work takes its place at the very head of all technical treatises on this subject of which there are several such in English though none have been published heretofore in German.

Dr. Martini's treatment is technical throughout. The book abounds in keys for determining the families, genera, and species of medical importance and serves well the purpose of reaching a precise determination regarding known arthropods which might be encountered in any particular case. The text is highly condensed and a veritable mine of detailed information; yet it is well written and the material is presented in attractive form. The illustrations are fairly numerous and altogether good although one cannot help wishing that the author had been able to present more. The completeness of the treatment might well be illustrated by the fact that one page for example carried figures from Riley and Johannsen opposite which one sees an illustration taken from sculpture at Nineveh. To some the work may appear too technical and it does serve a different and more indispensable purpose than the usual rather popular current review of a field of parasitology. One feels here confidence in the completeness and accuracy of the treatment accorded individual subjects even though minor defects appear from point to point. In most cases these are due to failure to consider recent publications which probably could not have been incorporated into the manuscript. Such conditions are unavoidable in a rapidly growing subject.

With all the good things that must and should be said of the work one cannot help expressing regret at the extreme transformation which certain well-known technical terms have suffered at the hands of the author. It is difficult to recognize the classical original in the form *Kutikula* and still more difficult to interpret *Zökum*. While the author is undoubtedly following a present day tendency in Germany a reviewer is nevertheless justified in passing criticism on him. Certain it is that German science can never hope to hold international rank if it insists upon such distortions of an ancient and honorable technical terminology. Dr. Martini's book merits high praise and will be found a valuable and necessary adjunct to the armamentarium of the parasitologist.

DIE WICHTIGSTEN PARASITISCHEN PROTOZOEN DES MENSCHEN UND DER TIERE. By WILHELM NÖLLER. Richard Schoetz, Berlin, 1922. 272 pp., 113 figures.

This constitutes Part I of the first volume of the treatise *Die tierischen Parasiten der Haus- und Nutztiere* by Ostertag, Wolffhügel and Nöller. The work is planned to give parasitologists a survey of those parasitic protozoa of practical importance and not to supplant or duplicate the more extended general texts on Protozoa already in existence. In contrast with such the general treatment given the group here is condensed whereas the data for individual forms of pathologic import are given more extended treatment. This has made the work indispensable to all who propose to determine precisely or investigate technically the pathogenic protozoa of man and the domestic animals.

The general section covers in brief but well-balanced form the structure and bionomics of Protozoa and includes a rich and extremely valuable treatment of the best modern methods for the investigation of these organisms both by cultures and in micro-preparations. The special part includes only the parasitic Rhizopoda, among which the author lists the Mycetozoa and a few other forms of doubtful position. After a condensed outline of the orders, families, and genera, each species is described in its ordinary and variant forms and its history recounted. Where necessary the differential diagnosis is discussed at length and the pathology, geographic distribution and therapy are equally fully treated. The author has brought together very completely the fragmentary and widely scattered studies on such parasites from hosts other than man and has compared them critically with the human species. In the main this critique is well done though opinions will differ as to the success achieved in individual cases and some American literature appears to be known only imperfectly and probably through abstracts merely.

The illustrations are abundant and peculiarly fine, no less than 113 text figures and 3 colored plates being used. The printer's skill, evident in the text as well, has achieved unusually good results in the figures and added materially to the attractiveness and usefulness of the book. The author emphasizes rightly the indispensable character of adequate references to the literature for those who undertake significant studies and apologizes for his evident inability to cover this field in this work. One gets some conception of the extent of the literature from the 30 odd pages of references on Parasitic Rhizopods alone which represent the limited though well-balanced and very useful selection which the author has given.

In so attractive and scholarly work it is difficult to justify on any grounds the use of such distorted spellings as "Kokzidien" for Coccidia. The language of science is international and while each people will naturally use its peculiar grammatical forms yet it appears as a strangely perverted nationalism to modify so radically the proper Latin orthography.

But there is little to criticize and the work should be highly commended both to special workers in this field and to the larger number who wish to secure an authoritative presentation of the current state of knowledge on these important organisms.

NEW HUMAN PARASITES

Tetranychina tuberculata Kishida 1921.—This mite found in human urine in Japan is distinguished from an American species, *T. harti* Ewing, by several characters, including the presence of two pairs of eyes, a transverse constriction of the body, and a dorsal shield. (Dobutsugaku Zasshi [Japanese text], 33: 438-443, 4 figs.; abstracted in Japan J. Zool., 1: 9.)

Spirochaeta sp. Salimbeni & Kermorgant 1923.—An organism considered provisionally as a spirochete is found in the blood of cases of measles only during the rise of fever prior to the eruption or during the first few hours, also in the urine during the descent of the fever. It may be cultivated anaerobically in symbiosis with a certain bacterium. It is very polymorphic in different cultural stages, varying from almost ultra-visible granulations in young cultures to spirals which may reach 300 microns in lengths. It has been kept alive for eight months in cultures, and appears to become better adapted to the culture medium with successive subinoculations. (Compt. rend. Acad. Sci., 177: 717-719.)

Leptospira couvyi Gomes de Faria 1923.—In a blood smear from a patient in Rio de Janeiro suspected of dengue 4 micro-organisms were found, in the form of an undulating filament, with slender extremities. In Leishman stain the organisms take a red color, almost purple. One or both of the extremities in the stained specimens are curved hook-like. In the middle portion of the filament there are numerous spirals, in addition to which the body presents two or three undulations. The length of the organism is 6 to 9 microns. Couvy, after whom the parasite is named, had previously reported the presence of spirochetes in the blood of dengue patients but did not describe them sufficiently to show whether his forms were the same as this. (Brazil-Med., 37: 287-288, 2 figs.)

Tetrachilomastix bengalensis Chatterjee 1923.—This flagellate is very commonly found in India in the stools of patients suffering from chronic intestinal complaints, but it appears to be non-pathogenic. A capacious cytostome differentiates it from Trichomonas. There is no axostyle. The presence of four flagella and an extra-cytostomic undulating membrane distinguishes it from *Chilomastix mesnili*. From *Tetrachilomastix intestinalis* it is distinguished by the presence of the extra-cytostomic membrane. Four types of the parasite may be made out in stained preparations: Large elongated forms, small oval forms, precystic stage, and cysts. (Indian J. Med. Res., 11: 177-180, pls. 12, 13.)